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Seed dormancy breaking in *Crataegus pedicellata*

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Abstract: The effects of stratification and scarification on seed dormancy breaking were compared in scarlet hawthorn (*Crataegus pedicellata* Sarg. = *C. coccinea* L). Ripe fruits were collected (in October) and the extracted nutlets were cleaned, and dried to a moisture content of 9–12%. Seed dormancy in this species was broken most effectively by warm-followed-by-cold stratification of nutlets, in a substrate or without any substrate, as well as at 15~25° or 20~30°C, i.e. with a cyclically alternating warm stage (16+8 hrs or 24+24 hrs/cycle) lasting 16–20 weeks, followed by the cold stage at 3°C lasting ca. 20 weeks, i.e. till the appearance of the first germinating seeds. After stratification, emergence rate is equally high (ca 76%) at cyclically alternating temperatures of 3~15°C or 3~20°C (16+8 hrs). Chemical scarification of nutlets in 96% sulphuric acid for 2 hrs, followed by warm-cold stratification at 20~30°/3°C, with a short, 4-weeks warm stage, also ensures a high emergence rate (85–93%). Seed desiccation (in nutlets) slowly to moisture content of 12–14%, after stratification in a substrate or scarification does not reduce the seedling emergence of seeds. Emergence decreased when seeds were desiccated after stratification without any substrate. Results provide new methods of breaking of dormancy and high germination and emergence of hard-coated *Crataegus* seeds in controlled conditions.

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

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Introduction

Seeds of many hawthorn species exhibit double dormancy: endocarp and embryo dormancy (Tyszkiewicz and Dąbrowska 1953, Nikolaeva 1967, Lang et al. 1987, Hartmann et al. 1997, Baskin and Baskin 2004). The basic procedure enabling dormancy breaking and thus germination is seed stratification in controlled conditions. In the case of physical causes of 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).

The genus *Crataegus* belongs to the family *Rosaceae*, subfamily *Maloideae*, which includes numerous and variable species. The great variability is caused by hybridization, polyploidization and apomixis (Dickinson and Phipps 1986, Phipps 1988, Radford et al. 1968, Robertson et al. 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. pedicellata*, 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). Scarlet hawthorn & Ontario hawthorn (*C. pediccelata* Sarg., syn. *C. coccinea* L.) is distributed in northeastern North America. In Poland it is an introduced species, commonly planted in parks (shrubs and trees 4–7 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 fruits are used in medicine to reduce blood pressure (Bugała 1991; Moerman 1998; Moore et al. 1986; McMillan-Browse 1985, Young and Young 1992).

The aims of this study were: to determine optimum conditions of seed dormancy breaking (stratification and/or chemical scarification); to identify the optimum temperature of germination and seedling emergence after dormancy breaking and the influence of drying after stratification the seeds on germination and seedling emergence rate

Material and methods

Fully ripe fruits of the scarlet hawthorn were collected in two locatities (Table 1) in October (2000–2003 years). 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 seeds 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) in a moist medium (sand and peat volume ratio 1:1, pH 3.5–4.5) or without any substrate (soaked only in water for 1 h once a week) and/or by scarification in concentrated sulphuric acid for 2 and 3 hrs followed by soaking in water for 24 hrs; all this in three replications of 50 seeds each. After the cold phase of stratification, the seeds were kept at 3~15°C or 3~20°C (16+8 hrs) and subjected separately 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. 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). 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, were given in captions to tables and figures. 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

Seed germination and seedling emergence from nutlets pretreated by 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 warm-cold stratification 15~25°C (16+8 hrs) lasting 16 weeks, followed by the cold phase at 3°C for 21 weeks; seeds germinated and emerged in cyclically

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

Seed lot no.	Provenance	Year of harvest	Moisture content of dried nutlets (%)	Seed viability* (%)
1	Poznań	2000	11.9	70.0
2	Poznań	2001	11.4	60.0
3	Poznań	2002	10.5	52.0
4	Szczepankowo	2002	8.8	40.0
5	Poznań	2003	9.8	68.0

*determinet by cutting test

Table 2. *Crataegus pediccelata* Sarg. Comparison of effectiveness of seed pretreatment by warm-cold stratification in a substrate. Experiment I, seed lot 1

Stratification			Temperature of the test		
warm phase	cold phase 3°C	germination	seedling emergence		
			3~20°C	3~15°C	3~20°C
°C	weeks	weeks	%	%	%
25°C	12	19	66	59	69
	16	21	61	58	54
mean			63	58	61
20~30°C	12	19	79	79	65
	16	20	79	80	76
mean			79	79	70
15~25°C	12	19	72	74	72
	16	21	81	80	83
	mean		76	77	77

*results related to full seeds only

Table 2 a. *Crataegus pedicellata* Sarg. Analysis of variance. Experiment I, seed lot 1

Source	DF	SS	F-test	Prob >F
Temperature of warm stratification A	2	804.17982	94.1185	<0.001
Time B	1	15.19706	3.5572	0.0714
A*B	2	189.30879	22.1561	<0.001
Temperature of the test C	1	10.19474	2.3863	0.1355
A*C	2	94.57279	11.0685	0.0004
B*C	1	0.50855	0.1190	0.7331
A*B*C	2	111.28018	13.0239	0.0001
error	24	102.5320	–	–

alternating temperature 3~15°C or 3~20°C (16+8 hrs) on average 81% (Tables 2, 2 a).

In Experiment II and III (seed lot 3 and 4) germination and seedling emergence were the highest after warm-cold stratification, with the warm phase at 20~30°C (24+24 hrs) and cold phase at 3°C. After stratification seedling emergence at 3~20°C (16+ 8 hrs) achieved high levels, i.e. 68% and 64% for both respectively (Tables 3, 4).

In experiment IV (seed lot 1), we compared the effectiveness of seed pretreatment by warm-followed-by-cold stratification at 15~25°/3°C (16+8 hrs) in a substrate or without any substrate. Best results of seedling emergence were achieved after warm-cold stratification at 15~25°/3°C (20+20 weeks) in any substrate – 54% (Table 5).

Table 3. *Crataegus pedicellata* Sarg. Comparison of effectiveness of seed pretreatment by warm-cold stratification in a substrate. *Experiment II, seed lot 3

Warm phase		Cold phase 3°C	Seedling emergence %
°C		weeks	
15~25°	16	16	66 a
20~30°			68 a
27.5°			55 c
30°			45 d
mean			58.5

*means followed by the same letters do not differ significantly at P<0.05, Tukey test

In experiment V (seed lot 3) highest level (68%) of seedling emergence at 3~20°C (16+8 hrs) was achieved after stratification in a substrate with the warm phase at 20~30°C (16+8 hrs) lasting 16 weeks, followed by the cold phase at 3°C lasting 20 weeks.

In this experiment differences in the warm stage of stratification in a substrate or without any substrate at 15~25°/3°C (16+8 h) did not have any effect on seed germination. Germination was similar in both variants: on average 66% and 62%, respectively. The use of constant temperature 30°C during the warm stratification phase turned out not effectively (Fig. 1).

Chemical scarification of seeds

Chemical scarification was tested in tree seeds lot (3, 4 and 5). Seedling emergence from seeds of *C. pedicellata* at 3~20°C was the highest (85%, 93% and 88% respectively) when the nutlets were scarified chemically for 2 hrs and next subjected to warm-cold phase stratification, with a 4-week warm phase at

Table 4. *Crataegus pedicellata* Sarg. Comparison of effectiveness of seed pretreatment by warm-cold stratification in a substrate. *Experiment III, seed lot 4

Warm phase		Cold phase 3°C	Seedling emergence %
°C		weeks	
15~25°			51 b
20~30°	16	24	64 a
30°			46 c
mean			53.6

Table 5. *Crataegus pedicellata* Sarg.Comparison of effectiveness of seed pretreatment by warm-cold stratification in a substrate or without any substrate.*Experiment IV, seed lot 1

	Stratification			Germination	Seedling emergence	
	warm phase		cold phase 3°C	3°C	3~15°C	3~20°C
	°C	weeks		%	%	%
in substrate	15~25°	16	20	34 e	33 e	40 cd
		20		31 e	38 e	48 b
	mean			32.5	35.5	44
without any substrate	15~25°	16	20	35 e	32 e	36 e
		20		34 e	42 c	54 a
	mean			34.5	37	45

Table 6. *Crataegus pedicellata* Sarg. Seedling emergence after scarification and warm-followed-by-cold stratification.* Experiment VI, seed lot 3, 4, 5

Seed lot	Seed pretreatment			Seedling emergence 3~20°C
	scarification	warm phase 20~30°C	cold phase 3°C	
no	hrs	weeks	weeks	%
3	2	4	18	85 b
	3			71 c
mean				78.0
4	2	4	27	93 a
	3			93 a
mean				93.0
5	2	4	20	88 b

*Information as in table 2 and 3

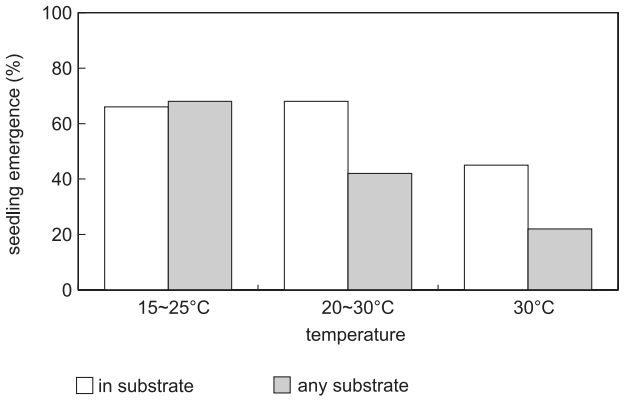

Fig. 1. *Crataegus pedicellata* Sarg. Comparison of effectiveness of seed pretreatment by warm-cold stratification in a substrate or without any substrate with the warm phase lasting 16 weeks and the cold phase lasting 18-22 weeks. Results related to full seeds only. Experiment V, seed lot 3

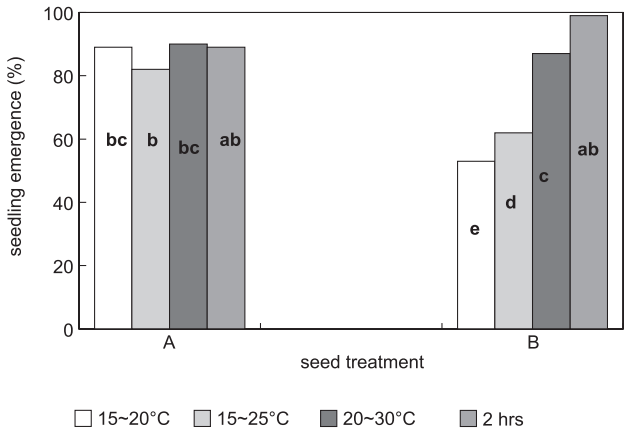

Fig. 2. *Crataegus pedicellata* Sarg. Seedling emergence at 3~20°C (16+8 hrs) mean seeds not dried (A) and dried (B) after warm-cold stratification of after scarification for 2 hrs followed by warm-cold stratification with a 4-week warm phase at 20~30°C (24+24 hrs). Means followed by the same letters do not differ significantly at $P<0.05$, Tukey test. Experiment VII, seed lot 5

20~30°C (16+8 hrs.) and cold phase at 3°C. The longer time of scarification (3 hrs) seems to be less effective (Table 6).

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

In Experiment VII (seed lot 5), slow drying of seeds after warm-cold stratification with the variable warm stage at 15~20°, 15~25° or 20~30°/3°C (24+24 hrs) caused a decrease in emergence rate. The mean seedling emergence of undried seeds was 87.0%, while that of dried seeds was 67.3%. Slow drying after warm-cold stratification in a substrate at 15~25°/3°C, caused a remarkable decrease in seedling emergence i.e. to 3%. After warm-cold stratification at 20~30°/3°C drying of stratified seeds to a moisture content of 10.8–12.3% caused a remarkable decrease in seedling emergence i.e. to 36.0%. After the scarification for 2 hrs followed by warm-cold stratification with a 4-week warm phase at 20~30°C (24+24 hrs) achieved high levels i.e. 89.0% while drying to a moisture content of ca. 12.0% after stratification did not reduce the seedling emergence. Seedling emergence was the highest 99.0% (Fig. 2).

Discussion

There exists some published information on seeds pretreatment in the scarlet hawthorn = *Crataegus pedicellata* Sarg.(McMillan-Browse 1985, Sheat 1948, Bird 1990). Recommended is warm-cold stratification of seeds at 15°C for 3 months and at 4°C for 3 months. Those findings were used for the planning of this study. However, for the scarlet hawthorn no data were available about seeds dormancy breaking. In this study, nutlets of the scarlet hawthorn were subjected to warm-followed-by-cold stratification with the warm phase at 25, 27,5 and 30°C or at cyclically alter-

nating temperature 15~25 or 20~30°C (16+8 hrs and 24+24 hrs) for 12, 16 and 20 weeks followed by the cold phase at 3°C for 16–20 weeks (until first radicles started to appear).

The results show that dormancy of seeds of the scarlet hawthorn can be optimally overcome by a long-lasting stratification in a moist medium in the cyclically alternating thermal regime 15~25° or 20~30°C, namely 16 weeks (16+8 hrs or 24+24 hrs) followed by 16–20 weeks at 3°C, i.e. to the time when the first radicles start to appear.

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 is important. In practice it is advisable to use the cycle 24+24 hrs (Tables 2, 3).The thermal conditions recommended by the authors mentioned above were less effective.

Our results indicate that seeds of *C. pedicellata* Sarg. can be successfully pretreated by stratification without any substrate, if the most favourable thermal variants of warm-cold stratification are applied (Table 5, Fig. 1). Such seed pretreatment in this species, not applied earlier, should be verified with the use of a large number of seed lots.

In our study, chemical scarification (for 2 or 3 hrs) 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 scarification of *C. monogyna* Jacq. in sulphuric acid such nutlets. (Piotto 2002).

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 the highest after 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 85%. After scarification for 2 hrs followed by warm-cold stratification with a 4-week warm stage at 20~30°C (24+24 hrs) high levels of seedling emergence i.e. 89,0% were achieved while drying to a moisture content of ca. 12.0% after stratification did not reduce emergence rate. The highest level of emergence was 99.0% (Fig. 2).

On the base of our earlier research that dormancy of seeds the hawthorns' species: *Crataegus monogyna* Jacq., *C. laevigata* L and *C. submollis* Sarg. can overcome by stratification in a moist medium first at 20~30° (24+24 hrs.) lasting 16 weeks followed by cold phase at 3°C for 16–20 weeks (Bujarska-Borkowska 2002, 2006, 2007).

Conclusions

Seed dormancy in the scarlet 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 16–20 weeks followed by the cold phase at 3°C, i.e. until first radicles start to appear (16–24 weeks).

Slow drying of seeds after stratification to moisture content of 9–13%, decreased emergence rates of seeds in comparison with undried seeds.

For *C. pedicellata* the most effective for breaking of dormancy was a chemical scarification of nutlets in 96% sulphuric acid for 2 hrs followed by a short, 4-week warm stage of stratification at 20~30°C (24+24 hrs.) and cold stratification at 3°C.

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