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SEED DORMANCY BREAKING OF WILD CHERRY (*PRUNUS AVIUM* L.)

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Abstract

Several methods for overcoming wild cherry seed dormancy have been studied. Wild cherry (*Prunus avium* L.) seeds have shown to be affected by combined dormancy: weak morphological and deep physiological dormancy. Fresh stones responded differently to the various methods for dormancy breaking. The study showed that the dormancy of fresh seeds is successfully overcome by sowing the seeds immediately after their harvest or in autumn. Stored seeds, however, did not have a positive response to these specific sowing periods. Acceptable germination rates were achieved after application of seed stratification. Findings showed that accelerated stratifications did not give positive results for successful dormancy break in the conditions of Bulgaria. Stored seeds should be sown in spring after application of combined warm and cold stratifications with the following durations: 6 weeks of warm period and 5 months of cold period.

Key words: germination, seed dormancy breaking, stratification, wild cherry.

Introduction

Seeds of wild cherry (*Prunus avium* L.) often do not possess satisfactory germination. It depends on the conditions during the year, the individual genetic differences and the embryo dormancy. This requires selecting a suitable sowing period and appropriate stratification (Michev 1953; Dirr and Heuser 1987; Suszka et al. 1996; Eşen et al. 2006, 2009).

Previous and some recent studies accepted that the stone endocarp caused the mechanical dormancy of seeds (Tukey 1924, Hartmann and Kester 1983, 2002; Çetinbaş and Koyuncu 2006). Different methods for its removal have been applied most often without positive results (Zielinski 1958). Later, Suszka et al. (1996) rejected the possibility of its role in germination delay.

It has been thought for many years that dormancy breaking requires cold stratification at temperatures from 1.7 °C to 6 °C (Crocker 1931, Giersbach and Crocker 1932, Coe and Gerber 1934, Haut 1938, Zielinski 1958, Huntzinger 1968, Seeley and Damavandy 1985).

Suszka (1967) proved that germination is significantly improved by alternating warm and cold stratifications: 14 days at tempera-

ture of 20 °C followed by 189 days at 3 °C. Similar approach was adopted by Dirr and Heuser (1987): 3–6 weeks of stratification at temperature of 15–21 °C followed by cold stratification at 4–5 °C for 12–21 weeks.

Suszka (1967) proposed a new method of stratification involving alternating treatments: initial period at temperature 25 °C followed by a cold period for two weeks at 3 °C and a subsequent period again at temperature of 25 °C (Table 1, test variant 1).

Later, some researchers also used pre-sowing preparatory treatments of alternating warm and cold phases. Muller et al. (1990) proposed 3 cycles of warm and cold stratification. Table 1 shows few more recommended approaches: Michalska and Suszka (1980a, 1980b) – test variant 2; Seeley and Damavandy (1985), Eşen et al. (2009) – test variant 3; Suszka et al. (1996) – test variants 4, 5, 6.

When the cold period is not long enough and is interrupted by a warm one, secondary dormancy is induced (John et al. 2003). This results in the epicotyl losing its ability to grow, the formation of a shortened shoot and leaf rosette, and a delay in the growth of rootlets (Suszka 1967, Huntzinger 1971, Michalska 1982).

Seeds may be sown immediately after harvest in July or August (Dirr and Heuser 1987, Suszka et al. 1996), or earlier in autumn, from the beginning of September to mid-October at the latest (Swingle 1925, Huntzinger 1971, Grisez 1974, Grzeskowiak et al. 1983). If the stones are stratified in a controlled environment, sowing may take place immediately before winter (Suszka et al. 1996).

Although the technology for seedling production is considered to be well-established, there are still unclear points concerning the organic dormancy and the ways for overcoming, which was proved by numerous varied recommendations for different pre-sowing treatments and periods of sowing. The reason is the regional nature of the studied biological traits. Presented issues outline the need for studies aiming to clarify the specificity of dormancy of native populations and the ways for overcoming it.

Material and Methods

Seeds from 6 sources were used in this study (Fig. 1):

Table 1. Test variants of pre-sowing treatments of wild cherry seeds.

Variant	Sequence/duration of stratification phases (weeks)											
	warm		cold		warm		cold		warm		Cold	
1	2 w	25 °C	2 w	3 °C	2 w	25 °C	12–16 w	3 °C	–		–	
2	2 w	20 °C	8 w	3 °C	2 w	25 °C	3 °C to the end		–		–	
3	2 w	20 °C	6 w	3 °C	2 w	25 °C	4 w	3 °C	2 w	25 °C	11 w	3 °C
4	–		6 w	3 °C	2 w	25 °C	2 w	3 °C	2 w	25 °C	12–16 w	3 °C
5	2 w	20 °C	6 w	3 °C	2 w	25 °C	2 w	3 °C	2 w	20–25 °C	8–12 w	3 °C
6	2 w	20–25 °C	2 w	3 °C	2 w	20–25 °C	12–16 w	3 °C	–		–	

Forest Service Targovishte – seed lots No 1, 2 (plot 13 и 187);

Forest Service Smyadovo – seed lots No 3, 4 (plot 156);

Forest Service Sofia – seed lot No 5 (plot 382);

Forest Service Gabrovo – seed lot No 6 (plot 257);

Forest Service Belovo – seed lot No 7 (plot 610);

Forest Service Cherni Lom – seed lot No 8 (plot 67).

The seeds were collected in 2006.

The seed viability has been assessed according to ISTA 1996.

The effect of sowing periods and various pre-sowing treatments on fresh and stored-for-one-year seeds has been studied to evaluate their germination in soil on the basis of the following test variants:

Variant 1 – Control: viability testing;

Variant 2 – Summer sowing in the field;

Variant 3 – Autumn sowing of stored seeds in the field;

Variant 4 – 4 months of cold stratification (according to ISTA 1996);

Variant 5 – Stratification: 3-months warm phase + 4-months cold phase;

Variant 6 – Stratification: 6-weeks warm phase + 5-months cold phase;

Variant 7 – Accelerated stratification: 6-weeks cold phase, 2-weeks warm phase, 2-weeks cold phase, 2-weeks warm phase, 12 to 16-weeks cold phase;

Variant 8 – Accelerated stratification: 2-weeks warm phase; 2-weeks cold phase, 2-weeks warm phase, 2-weeks

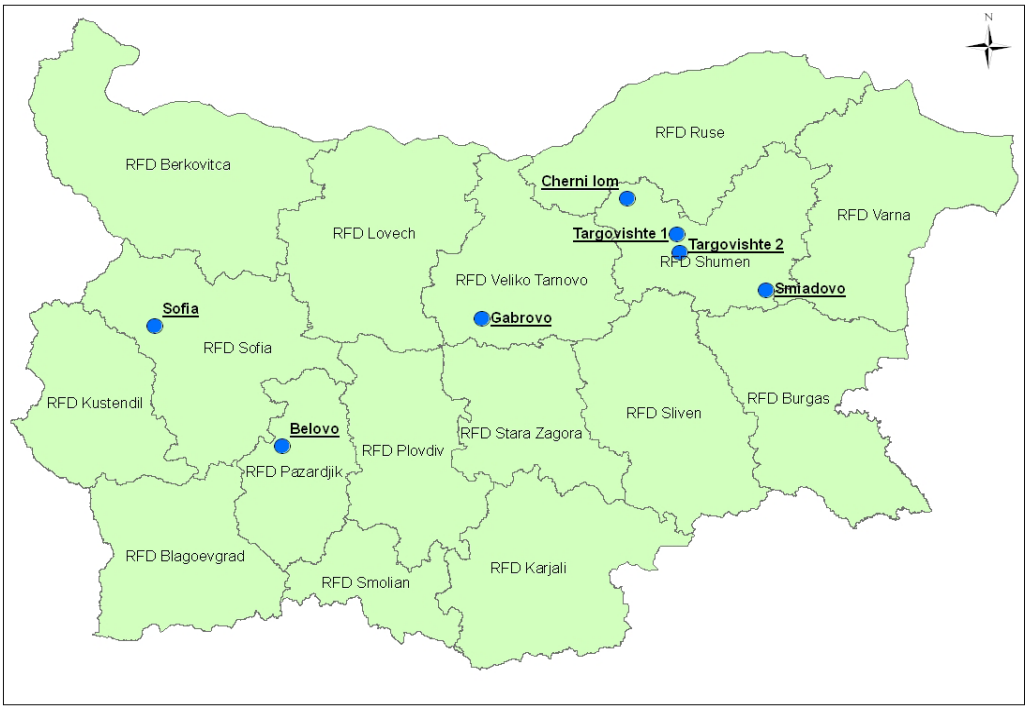

Fig. 1. Map of Bulgaria with the sources of seeds used in the experimental work.

cold phase, 2-weeks warm phase, 8-weeks cold phase.

The medium used for the pre-sowing treatments was perlite. Sowing took place in a greenhouse at temperature of 20–25 °C.

Results were statistically analyzed using SPSS software, ANOVA and Post Hoc Test and a confidence level at 95 %. To show the statistical differences of the results, the test variants without statistically significant differences were indicated with the same capital letter, while those with statistically significant differences – with a different letter.

Results and Discussion

The seed viability (Table 2) varied from 80 % (Sofia) to 98 % (Smyadovo). After one year of storage it decreased by between 1 and 5 % depending on the seed lot.

The seed germination is shown in Table 2. Fresh seeds had the highest germination in the summer and autumn sowing test variants (62 % on average). Values for each seed lot vary from 51 % (Sofia) to 73 % (Targovishte) for the summer sowing test variant, and from 42 % (Belovo) to 78 % (Targovishte) for the autumn sowing one.

Table 2. Seed viability.

No	Seed lot	Variant 1	Variant 2		Variant 3		Variant 4		Variant 5		Variant 6		Variant 7		Variant 8	
		Seed viability, %	M±SE	V, %	M±SE	V, %	M±SE	V, %	M±SE	V, %	M±SE	V, %	M±SE	V, %	M±SE	V, %
1	Targovishte 1	88	56±3	32.9	61±4	57.7	6±0.3	0.3	5±1	1.7	16±2	13.3	1±0.3	0.3	10±2	17.7
2	Targovishte 1s	93	–	–	7±1	8.7	5±3	25.7	19±6	126.2	82±3	30.2	2±1	8.2	5±3	27
3	Targovishte 2	97	73±3	44.7	78±4	76	9±2	24.9	10±3	24.9	47±6	165.7	1±0.5	0.9	31±4	65.6
4	Targovishte 2s	97	–	–	2±2	14.9	21±2	22.9	79±2	10.9	49±10	375.6	0	0	0	0
5	Smyadovo 1	98	59±7	208.2	71±2	12.2	13±3	41	11±3	30.3	16±2	10.9	2±1	8	36±3	32.9
6	Smyadovo 1s	31	–	–	0	0	0	0	0	0	10±0.5	0.9	0	0	0	0
7	Smyadovo 2	98	58±8	235	68±3	30.9	12±4	49.3	13±2	14.9	16±2	9.6	2±1	4.7	20±6	127
8	Smyadovo 2s	96	–	–	1±1	2	34±3	25.6	77±1	6.7	82±5	117.6	1±1	1	0	0
9	Belovo	95	67±6	157.3	42±1	4.9	5±1	6.7	4±0.4	0.7	8±1	5.7	1±05	0.9	11±3	30
10	Belovo – s	93	–	–	22±2	13.3	16±2	21.6	72±5	88.7	73±5	86.7	4±2	12.3	6±2	18.9
11	Sofia	80	51±5	106.9	49±0.5	0.9	9±0.3	0.3	2±0.4	0.7	12±3	34.7	2±1	1.7	12±1	5.67
12	Sofia – s	75	–	–	14±3	37.6	24±3	34.9	66±7	199	71±6	140.7	2±1	6.9	4±2	18.9
13	Gabrovo	97	63±3	39.6	61±1	4.3	18±1	2.7	5±2	13.3	15±3	43.3	3±1	3.3	21±1	4.3
14	Gabrovo – s	96	–	–	1±0.3	0.3	3±1	1.7	84±2	14	82±6	155.3	3±1	5.7	0	0
15	Cherni Lom	98	72±4	55.3	63±2	24.9	17±2	13.3	18±1	7	10±5	87	2±1	1.7	11±2	12.2
16	Cherni Lom – s	97	–	–	1±1	2	29±3	48.2	32±7	211.6	72±5	116.3	1±1	2.2	0	0

Note: Used symbols have following meaning:
1. letter “s” is used to indicate stored seeds of various seed lots.
2. “M” – mean values; “SE” – Standard error; “V, %” – coefficient of variation.

The autumn sowing test variant showed the same or higher germination percentage compared to that of the summer sowing. This indicates that the morphological dormancy was not too pronounced, and the period of seed dormancy was taking place at the higher temperatures in autumn before the winter cold was enough for the dormancy break.

The cold stratification for 4 months resulted in low germination: 11 % on average, from 5 % (Belovo) to 18 % (Gabrovo). This is an indicator of morphological dormancy, which requires a warm phase.

The test variants of warm and cold stratification, however, did not have the expected high germination percentage of the fresh seeds. Test variant 5 had an average value of 8 %: from 2 % (Sofia) to 18 % (Cherni Lom). Test variant 6 resulted in an average germination of 17 %, varying from 8 % (Belovo) to 47 % (Targovishte). These results indicate that wild cherry seeds do possess combined morpho-physiological dormancy. It can be accepted that a warm phase for 6 weeks is a long enough period. At the same time, a 4-month-long cold period was not long enough to overcome the physiological dormancy and better results were obtained when the cold phase lasts for at least 5 months.

The alternation of short, warm and cold phases (test variant 7) probably led to the induction of secondary seed dormancy. The low germination indicates that when there is morphological dormancy, it is not justified to begin the pre-sowing treatment with a cold phase.

Having a warm phase for a period of 2 weeks at the start and increasing the number of treatments (test variant 8) resulted in increase in germination from 2 % to 19% (from 11 % for Belovo and Cherni Lom to 36 % for Smyadovo 1).

Stored seeds showed different tendencies in comparison with fresh seeds. Seeds from all seed lots did not germinate in the summer sowing test (Table 2). The autumn sowing also showed very low values: an average of 6 %, from 1 % (Smyadovo 2, Gabrovo and Cherni Lom) to 22 % (Belovo).

In general, common warm and cold stratifications have shown considerably better results in comparison with the accelerated pre-sowing treatments.

The stored seeds' germination was most intensive with the warm and cold stratification (test variant 6) – on average 61 %, from 49 % (Targovishte 2) to 82 % (Gabrovo, Smyadovo 2 and Targovishte 1). An exception with a low germination percentage is the seed lot of Smyadovo 1.

Test variant 5 had an average germination percentage of 54 %. The germination percentage of the seed lots ranged from 19 % (Targovishte) to 84 % (Gabrovo).

Accelerated stratifications had a seed germination of less than 10 %. Considering the fact that the 4-months-long cold stratification led to an average germination of 16 % (from 3 % for Gabrovo to 34 % for Smyadovo 2), it can be concluded that these accelerated stratifications induced secondary physiological dormancy in the stored seeds as well.

Table 3 shows the significance of various factors on seed germination. Of the three tested factors, type of stratification/sowing period and seed lot quality significantly affected the seed germination.

Of the two factors, a stronger effect on seed germination had the type of stratification (92 % in the fresh seeds, and 77 % in the stored seeds). The seed lot quality had a considerably less profound effect: 7 % in the fresh seeds and 22 % in the

stored seeds, respectively. A factor without statistical significance was the type of seeds. This means that regardless of whether the seeds are fresh or stored, selecting the right approach for dormancy breaking, i.e. type of stratification or sowing period, ensures high soil germination.

Table 3. Effect of various factors on seed germination (ANOVA test).

Factor	Degrees of freedom	Level of significance (p-value)
Type of stratification	7	0.000
Seed lot	7	0.000
Type of seeds (stored/fresh)	1	0.395
Type of stratification * seed lot	49	0.000
Type of stratification * Type of seeds	7	0.000
Seed lot * Type of seeds	7	0.000
Type of stratification * Type of seeds * Seed lot	42	0.000

Tables 4 and 5 show the significance of the different factors affecting seed germination.

Considering the method applied for overcoming the organic dormancy in the process of seed germination, it has been found out that summer and autumn sowing of the fresh seeds led to statistically insignificant results: 62 %.

Statistically insignificant results were also obtained for the stored seeds from the stratification test variants 4 and 5: from 11 % to 8 %. These results show that the period of 4 months is not long enough cold phase.

Statistically, test variants 6 and 7 can be regarded as insignificant: their germinated seeds were 17 % and 19 %, respectively.

Test variant 7 had the lowest germination of 2 % which is statistically significant in comparison to all other test variants. Therefore, in order to overcome the morpho-physiological dormancy, it is not appropriate to begin the pre-sowing treatment with a cold phase.

For the stored seeds, only the two test variants of accelerated stratification had statistically insignificant results: germination of 2 %. All other test variants show values which have statistically significant differences.

The highest germination of 61% was obtained with the warm and cold stratification of test variant 6.

Table 4. Effect of type of stratification on seed germination, %.

Type of stratification	Fresh seeds		Stored seeds	
	M±SE	V, %	M±SE	V, %
Seed viability	94±1 a	38.6	85 ±4 a	47.4
Summer sowing	62±2 b	140.8	–	–
Autumn sowing	62±2 b	140.2	6±1 b	65.4
Variant 4	11±1 c	34.9	16±2 c	165.9
Variant 5	8±1 c	34.2	54±6 d	986.6
Variant 6	17±2 d	171.2	61±5 e	738.8
Variant 7	2±0 e	2.7	1±0 f	5.2
Variant 8	19±2 d	119.3	2±0 f	12.1

Table 5. Effect of seed lot quality on seed germination, %.

Seed lot	Fresh seeds		Stored seeds		Statistical significance
	M±SE	V, %	M±SE	V, %	
Smyadovo 1	38±6	110.8	6±2	012.1	A
Smyadovo 2	36±6	111.6	37±7	152.2	B
Targovishte 1	30±6	100.4	30±7	141.7	C
Targovishte 2	43±6	122.8	35±7	148.6	D
Gabrovo	36±6	105.4	38±8	191.4	B
Belovo	29±6	115.3	41±7	125.5	B
Sofia	27±5	78.5	37±6	95.6	C
Cherni Lom	36±6	118.9	33±6	134.6	B

The summer and autumn sowing of fresh seeds (Table 4) led to the same results with regard to germination as did the application of the warm and cold stratification for the stored seeds: germination of 62 % and 61 %, respectively.

Table 5 shows the effect of seed lot quality on seed germination. Used seed lots were not uniform, and they showed different physiological response to the various treatments. These may be grouped as follows: the first group with the highest average germination of 43 % includes seed lot Targovishte 2. The second group includes seed lot Smyadovo 1 (38 %). The third group consists of the following seed lots: Smyadovo 2, Gabrovo, Belovo and Popovo with an average germination ranging between 29 % and 36 %. The seed lots of low quality seeds are Sofia and Targovishte 1 with an average germination ranging between 27 % and 30 %.

After storing the seeds for one year, their germination decreased with statistical significance by 2 % on average, from 34±2 % for fresh seeds to 32±2 % for stored seeds.

Conclusion

Wild cherry seeds possess combined dormancy: relatively weak morphological and deep physiological dormancy.

The storage of seeds until maturity at the beginning of September following the rules for storing dry seeds does not intensify their typical organic dormancy.

Fresh and stored seeds respond differently to the various approaches for overcoming their organic dormancy. Stratification of fresh seeds does not successfully overcome dormancy. The best approach for them is to be sown in summer, immediately after being harvested, or at the beginning of September.

Stored seeds do not have a positive response to specific periods of sowing, whether in summer or autumn. Good germination for them can be expected after applying a pre-sowing treatment. The accelerated combined stratifications do not realize the seeds' biological germination potential in the conditions of Bulgaria. Stored-for-one-year seeds should be sown in spring after warm and cold stratification involving a 6-week-long warm phase and a 5-month-long cold phase.

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