



Seed germination of *Crataegus monogyna* – a species with a stony endocarp

Abdenour Kheloufi¹✉, Lahouaria Mounia Mansouri¹, Cécile Vanbellinghen²

¹Department of Ecology and Environment, University of Batna2, Batna, Algeria

²Department of Quality and Animal Welfare, Seed Testing Laboratory, Chemin de Liroux, Gembloux, Belgium

✉ abdenour.kheloufi@yahoo.fr

ARTICLE INFO

Citation:

Kheloufi A, Mansouri LM, Vanbellinghen C (2019) Seed germination of *Crataegus monogyna* – a species with a stony endocarp. *Reforesta* 7: 73-80.

DOI: <http://dx.doi.org/10.21750/REFOR.7.06.68>

Editor: Vladan Ivetić, Serbia

Received: 2019-06-16

Accepted: 2019-06-22

Published: 2019-06-28



Copyright: © 2019 Kheloufi Abdenour, Mansouri Lahouaria Mounia, Vanbellinghen Cécile. This work is licensed under a [Creative Commons Attribution 4.0 International Public License](https://creativecommons.org/licenses/by/4.0/).



Abstract

The present work demonstrates the effects of moist cold stratification on seed dormancy breaking in Hawthorn (*Crataegus monogyna* Jacq.). We also examined the fruit and seed morphology. Mature and ripe fruits were collected, the pulp removed and the seeds (stones) left in the sun to dry for three days. Four temperature regimes viz. 4°C, 6.5°C (natural conditions where the fruits were harvested), 10°C and 20°C were used for stimulating seed germination under total darkness. For each treatment, there were four replicates with 50 seeds incubated in a plastic container between two layers of moist sand at 15%. At the end of the experiment, non-germinating seeds were tested for viability using Tetrazolium chloride (TZ). After 4 months, the final germination (FGP) was expressed as a percentage of the total number of seeds in each treatment. The fruits of Hawthorn were 14.9 ± 0.73 mm long and 15.1 ± 0.84 mm in diameter and weigh 2.05 ± 0.28 g. The seeds were 8.29 ± 0.43 mm long and 6.75 ± 0.39 mm in diameter and weigh (0.25 ± 0.04) g. The thousand-fruit weight was 2,000 g and of the thousand-seeds weight was 280 g. The statistical analysis indicated significant effect ($p < 0.0001$) of treatment on seed germination. Dormancy in this species was broken most effectively by cold stratification at 4°C and under natural conditions with 76% and 67.5% of FGP, respectively. Most of the nongerminated seeds of *C. monogyna* were viable (dormant) as judged by TZ.

Keywords

Hawthorn; Stratification; Germination; Seedling emergence; Storage

Contents

1	Introduction	73
2	Materials and methods	74
3	Results and discussion	76
3.1	Morphometric diversity between fruits and seeds	76
3.2	Seed germination and viability	76
4	Conclusion	78
5	Acknowledgment	78
6	References	78

1 Introduction

The ability to germinate under a variety of environmental conditions is essential for plant species inhabiting a wide range of altitudes and latitudes (Cavieres and Arroyo 2000). Seeds represent the principal form of dissemination for most plant species and are largely responsible for the preservation of biodiversity (Marcos Filho 2005). Seed germination is influenced by temperature regimes, which act directly on the velocity of water absorption and on biochemical reactions, thus exercising a determinant role in germination processes (Stokes 1965; Roberts 1988; Xia et al. 2016). Most forest seeds possess a hard tegument that is impervious to water, causing dormancy (Farmer 2017). Indeed, there are different types of dormancy: exogenous (determined by the properties of the seed coat); endogenous (determined by the physiological state of the embryo) and combined (determined by a combination of the two previous factors) (Aragón-Gastélum et al. 2018). In the case of embryonic dormancy, the inhibition of germination is due to the action of certain plant hormones (Shu et al. 2016). The hardness of this dormancy differs from one species to another (Lan et al. 2018). The effect of temperature is the main treatment to break this dormancy by using different modes of freezing and alternating temperatures (Bujarska-Borkowska 2002). These seeds often take a very long time to germinate, resulting in heterogeneity in the seedlings, an inconvenience for reforestation success. It is therefore often necessary to apply a treatment before sowing, to assure high, fast and uniform germination (Kheloufi 2017; Kheloufi et al. 2018).

The genus *Crataegus* belongs to the family Rosaceae, subfamily Amygdaloideae, which includes very numerous and variable species (GRIN-Taxonomy 2019). Hawthorn (*Crataegus monogyna* Jacq.) usually grows into a big or a small tree up to 8 m height. Fruits are spherical or egg-shaped, containing 1 and rarely 2 stones (Bujarska-Borkowska 2002). Hawthorn trees tend to be long-lived (Norris 2005). The fruits are vermilion red and perfectly edible but bland and floury (Özcan et al. 2005). They are especially a good food for birds during winter months (Sallabanks 1992). *C. monogyna* is a species that adapts to several types of soil. However, it prefers sufficiently cool clay-limestone soils (Snow et al. 1997). It is also a valuable therapeutic plant (Graf et al. 2010). The flowers are used to produce medicines affecting heart and nerves and lowering blood pressure (Rigelsky and Sweet 2002).

In light of the general lack of detailed information about germination and seedlings production in Hawthorn, the present study evaluated the seed germination behavior of the stony endocarp seeds under different conditions of moist cold stratification and describes the fruit and the seed morphology. On the other hand, the viability of nongerminated seeds was tested at the end of the experiment using Tetrazolium Chloride.

2 Material and methods

The seeds of Hawthorn used in the present experiments were obtained from freshly mature and ripe fruits harvested from five trees growing at 1630 meters of altitude in an Apple Orchard which is located in Theniet El Abed (Batna, Algeria) (Latitude: 35°20'12.8"N ; Longitude 6°20'39.97"E). These trees are over 20 years old and about 6 m height. The fruit sample was obtained by mixing the fruits of the five trees. After harvesting, the seeds (stones) were extracted by opening the fruits and removing the pulp using running water. A total of 100 fruits and 100 seeds were used

for biometric determinations using a digital caliper (Figure 1). The length is a distance between the base and the apex and the diameter in the median region of both fruits and seeds. The fruit and seed weights were also estimated on basis of the weight of the 100 unit samples.

Four temperature regimes *viz.* 4°C, 6.5°C (natural conditions where the fruits were harvested), 10°C and 20°C were used for stimulating seed germination under total darkness. For the natural conditions, the weather station of Batna (Indicative: 60468, DABT, Algeria) recorded the following values (Min temperature: -1.1°C; Max temperature: 16.6°C; Average temperature: 6.5°C) for the 4 months of the experiment (December 2018 – March 2019) at 1,052 m of elevation.

For each treatment, there were four replicates with 50 seeds incubated in a plastic container (17 cm Length x 12 cm Height x 10 cm Width) between two layers of moist sand at 15%. At the end of the experiment, nongerminating seeds were tested for viability using Tetrazolium chloride (TZ) as described by ISTA (2003). Stones were bisected transversely (1/3 from distal end) to remove a small proportion of the seed coat before immersing in a solution of 1% buffered 2,3,5-Triphenyl Tetrazolium Chloride. Immersed seeds were then incubated in the dark for 18 hours at 30°C before being evaluated for staining, indicating respiring tissue, under a microscope. Vital structures within the seed were evaluated based on staining intensity as fully stained (viable), unstained (non-viable) or partially stained (interpreted as viable or non-viable, depending on the intensity and location of staining).



Figure 1. *Crataegus monogyna* tree, fruits, seeds, and flowers.

After four months, the final germination (FGP) was expressed as a percentage of the total number of seeds in each treatment. Seeds were counted as germinated when the radicle growth reached ≥ 2 mm. Differences between treatments after ANOVAs were carried out through mean comparison contrasts. Multiple comparisons of means were performed with Duncan's test ($\alpha = 0.05$). All statistical methods were performed using SAS Version 9.0 (Statistical Analysis System) (2002) software.

3 Result and discussion

3.1 Morphometric diversity between fruits and seeds

According to Table 1 the fruits of Hawthorn were 14.9 ± 0.73 mm long and (15.1 ± 0.84) mm in diameter and weigh 2.05 ± 0.28 g. The seed were 8.29 ± 0.43 mm long and 6.75 ± 0.39 mm in diameter and weigh 0.25 ± 0.04 g. The thousand-fruit weight was 2,000 g and of the thousand-seed weights the same number of seeds weigh 280 g.

Table 1. Fruits and seeds characteristics of *Crataegus monogyna* (n=100).

	Fruits	Seeds
Weight (g)	2.05 ± 0.28	0.25 ± 0.04
Length (mm)	14.9 ± 0.73	8.29 ± 0.43
Diameter (mm)	15.1 ± 0.84	6.75 ± 0.39
1000-sample weight (g)	2000	280

3.2 Seed germination and viability

At each stratification period, final germination under 4°C and 6.5°C of moist stratification was significantly higher than germination with 10°C and 20°C ($p < 0.0001$). Under the first two thermal regimes, the stratification period was necessary to initiate seed germination and reach a maximum germination of 76% and 67.5%, respectively (Table 2, Figure 2). No germination was observed for the seeds treated at 20°C after the four-month period. However, the seeds treated with 10°C of moist stratification enhanced the germination but with a low rate of 7% FGP (Table 2).

Table 2. Final germination percentage (FGP) and Viability Test in Tetrazolium (TZ) in Hawthorn seeds after four months of incubation. The same alphabet along the column indicates no significance difference (Duncan Multiple Range Test) (n = 4 × 50 seeds).

Cold stratification Treatment	FGP (%)	Viability Test in Tetrazolium (TZ)	
		Viable (Dormant)	Non-viable (Dead)
Germination chamber (20 °C)	0.00 ± 0.00^D	86.0 ± 4.89^A	14.0 ± 4.89^A
Germination chamber (10 °C)	7.00 ± 2.58^C	88.5 ± 3.00^A	4.50 ± 1.00^B
Natural conditions (6.5°C)	67.5 ± 3.41^B	26.5 ± 3.41^C	6.00 ± 1.63^B
Germination chamber (4 °C)	76.0 ± 1.63^A	19.0 ± 2.00^D	5.00 ± 1.15^B
<i>F of Fisher</i>	1198.4	459.3	10.9
<i>p</i>	< 0.0001	< 0.0001	< 0.0001

According to our results, most of the nongerminated seeds of *C. monogyna* were alive (Dormant) as judged by TZ (Table 2). These results showed that the seeds

treated with 10°C and 20°C remain viable, waiting for the favorable conditions to break the dormancy. On the other hand, those treated with 4°C and 6.5°C will need more time to reach the maximum of germination under these thermal conditions. According to the same table, the rate of non-viable seeds was higher in seeds incubated at 20°C, reaching 14% against (4-6%) recorded in other treatments.

A lot of forest species are abandoned because of difficulty with seed germination and establishment (Kheloufi 2017). Indeed, there are few studies concerning the seed pretreatments of Hawthorn to germination. Several results suggest that Hawthorn seeds are unable to germinate during autumn, so they get into a dormancy process to avoid harsh winter conditions and germinate after snowmelt in early summer (Bujarska-Borkowska 2002). This fact has been interpreted by different authors as a maturation period to complete development of embryos (Hilhorst 2010). Our results showed that in the natural condition (1,630 m of elevation) or under germination chamber conditions of 4°C, freshly seeds were able to germinate after 4 months.



Figure 2. Seedlings propagated from Hawthorn seeds.

Seed germination is influenced by storage duration and internal factors controlling dormancy, including phytohormones (abscisic acid) inducing dormancy, and by seed coat factors (Bewley, 1997). Cold stratification is known to improve germination percentage in many *Crataegus* species (Morgenson 2000). Table 3 summarizes some previous results on the germination improvement in *C. monogyna*. Authors proved effective induction of *C. monogyna* seed germination by means of cold-wet stratification, with longer treatment periods required for populations from higher elevations.

Table 3. Results of research and various suggestions for the seed germination of *Crataegus monogyna*.

Treatment	Authors
Treating stones with concentrated sulphuric acid for 2 hours, and then subjecting them to cold stratification.	Holmes and Buszewicz (1958)
Stratifying seeds for 5 months at 25°C and then for the next 5 months at 5°C.	Nyholm (1975)
Stratifying seeds 3-5°C after 180 days.	Bärtels (1982)
Warm-followed-by-cold stratification at 20-25°C for 4-8 weeks, and later at 1-5°C for 12-16 weeks is also recommended.	Gordon and Rowe (1982)
Treatment with concentrated sulphuric acid and then sowing is recommended.	St.-John (1983)
The use of warm-cold stratification, first at 25°C for 3 months, then the cold one at 3°C for 9 months, and next 35 days at 20°C in sand.	ISTA (1999)
Seeds germinate vigorously (in 3-5 weeks) and at a high percentage at temperatures of 3~10°C.	Bujarska-Borkowska (2002)
Incubate in moist substrate (sand) 3 months at 25°C followed by 9 months prechill.	ISTA (2019)

4 Conclusion

Understanding of germination requirements is crucial for regeneration and successful tree establishment in forest nurseries as well as for direct plantation, where, during the last decades, there has been a rapid loss in natural plant cover associated with an erosion of the biodiversity. A compilation of the literature relating to the germination of *C. monogyna* indicates that optimal temperature conditions for seed germination could result from variations in their physiological dormancy levels that are directly related to seed collection and storage. We suggest the use of freshly mature and ripe fruits to extract the seeds for the production of young seedlings.

5 Acknowledgment

Authors are grateful to Zeroual Ismail (PhD student in Plant ecophysiology) for giving us access to his Apple orchard for harvesting Hawthorn fruits.

6 References

- Aragón-Gastélum JL, Flores J, Jurado E, Ramírez-Tobías HM, Robles-Díaz E, Rodas-Ortiz JP, Yáñez-Espinosa L (2018) Potential impact of global warming on seed bank, dormancy and germination of three succulent species from the Chihuahuan Desert. *Seed Sci Res* 28(4):312-318. <https://doi.org/10.1017/S0960258518000302>
- Bärtels A (1982) *Rozmnażanie drzew i krzewów ozdobnych* (Propagation of ornamental trees and shrubs). PWRiL, Warszawa (in Polish).
- Bewley JD (1997) Seed germination and dormancy. *The Plant Cell* 9(7):1055. <https://doi.org/10.1105/tpc.9.7.1055>
- Bujarska-Borkowska B (2002) Breaking of seed dormancy, germination and seedling emergence of the common hawthorn (*Crataegus monogyna* Jacq.). *Dendrobiology* 47:61-70.
- Cavieres LA, Arroyo, MT (2000). Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae) - altitudinal variation in the Mediterranean Andes of central Chile. *Plant Ecol* 149(1):1-8.

- Cavieres LA, Sierra-Almeida A (2018) Assessing the importance of cold-stratification for seed germination in alpine plant species of the High-Andes of central Chile. *Perspectives in Plant Ecology, Evolution and Systematics* 30:125-131. <https://doi.org/10.1016/j.ppees.2017.09.005>
- Farmer R (2017) *Seed ecophysiology of temperate and boreal zone forest trees*. Routledge, 1st Edition, 257p. <https://doi.org/10.1201/9780203740057-1>
- Gordon AG, Rowe DCF (1982) *Seed manual for ornamental trees and shrubs*. Stationery Office, Agronomy, 132p.
- Graf BL, Raskin I, Cefalu WT, Ribnicki DM (2010) Plant-derived therapeutics for the treatment of metabolic syndrome. *Curr Opin Invest Dr* 11(10):1107-1115.
- GRIN-Taxonomy (2019) Germplasm Resources Information Network. U.S. National Seed Herbarium, Agricultural Research Service, National Plant Germplasm System. <https://www.ars-grin.gov>
- Hilhorst HWM (2010) Standardizing seed dormancy research. In: Kermod AR, editor. *Seed dormancy: methods and protocols*. *Methods in Molecular Biology* 773:43-52. https://doi.org/10.1007/978-1-61779-231-1_3
- Holmes GD, and Buszewicz G (1958) The storage of seed of temperate forest tree species. *Forestry Abstracts* 19:313-322.
- ISTA (International Seed Testing Association) (1999) *Seed Science and Technology*, 27, Supplement.
- ISTA (International Seed Testing Association) (2003) *ISTA Working Sheets on Tetrazolium Testing*. Volume II: Tree and Shrub Species. (eds Leist N, Kramer S and Jonitz A). International Seed Testing Association, Bassersdorf, Switzerland.
- ISTA (International Seed Testing Association) (2019) *International Rules for Seed Testing*, chapter 5: The germination test, 5-34, Bassersdorf, CH. <https://doi.org/10.15258/istarules.2019.05>
- Kheloufi A (2017) Germination of seeds from two leguminous trees (*Acacia karroo* and *Gleditsia triacanthos*) following different pre-treatments. *Seed Sci Technol* 45(1):259-262. <https://doi.org/10.15258/sst.2017.45.1.21>
- Kheloufi A, Mansouri L, Aziz N, Sahnoune M, Boukemiche S, Ababsa B (2018) Breaking seed coat dormancy of six tree species. *Reforesta* (5):4-14. <https://doi.org/10.21750/REFOR.5.02.48>
- Lan Q, Yin S, He H, Tan Y, Liu Q, Xia Y, When B, Baskin JM (2018) Seed dormancy-life form profile for 358 species from the Xishuangbanna seasonal tropical rainforest, Yunnan Province, China compared to world database. *Sci Rep-UK* 8(1):4674. <https://doi.org/10.1038/s41598-018-22930-5>
- Marcos Filho J (2005) *Fisiologia de sementes de plantas cultivadas*. Piracicaba: FEALQ, 495p (in Portuguese).
- Morgenson G (2000) Effects of cold stratification, warm-cold stratification, and acid scarification on seed germination of 3 *Crataegus* species. *Tree Planters Notes* 49(3) :72-74.
- Norris JE (2005) Root reinforcement by hawthorn and oak roots on a highway cut-slope in Southern England. *Plant Soil* 278(1-2):43-53. <https://doi.org/10.1007/s11104-005-1301-0>
- Nyholm J (1975) Germination of tree seeds. Dormancy. *Acta Horticulture* 54:21-24. <https://doi.org/10.17660/ActaHortic.1975.54.2>
- Özcan M, Haciseferoğulları H, Marakoğlu T, Arslan D (2005) Hawthorn (*Crataegus* spp.) fruit: some physical and chemical properties. *J Food Eng* 69(4):409-413. <https://doi.org/10.1016/j.jfoodeng.2004.08.032>
- Rigelsky JM, Sweet BV (2002) Hawthorn: pharmacology and therapeutic uses. *Am J Health-Syst Ph* 59(5):417-422. <https://doi.org/10.1093/ajhp/59.5.417>
- Roberts EH (1988) Temperature and seed germination. In *Symposia of the Society for Experimental Biology* 42:109-132.
- Sallabanks R (1992) Fruit fate, frugivory, and fruit characteristics: a study of the hawthorn, *Crataegus monogyna* (Rosaceae). *Oecologia* 91(2):296-304. <https://doi.org/10.1007/BF00317800>
- Shu K, Liu XD, Xie Q, He ZH (2016) Two faces of one seed: hormonal regulation of dormancy and germination. *Mol Plant* 9(1):34-45. <https://doi.org/10.1016/j.molp.2015.08.010>
- Snow CSR, Marrs RH, Merrick L (1997) Trends in soil chemistry and floristics associated with the establishment of a low-input meadow system on an arable clay soil in Essex. *Biol Conserv* 79(1):35-41. [https://doi.org/10.1016/S0006-3207\(96\)00103-6](https://doi.org/10.1016/S0006-3207(96)00103-6)

- St-John S (1982) Acid treatment of seeds of *Crataegus monogyna* and other *Crataegus species*. Combined Proceedings, International Plant Propagators Society 32: 203-205.
- Stokes P (1965) Temperature and seed dormancy. In *Differenzierung und Entwicklung/Differentiation and Development* (pp. 2393-2450). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-50088-6_60
- Xia Q, Ando M, Seiwa K (2016) Interaction of seed size with light quality and temperature regimes as germination cues in 10 temperate pioneer tree species. *Funct Ecol* 30(6):866-874. <https://doi.org/10.1111/1365-2435.12584>