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SEED VIABILITY AND MOISTURE CONDITION OF DISPERSED SEEDS, OF FIVE TEMPERATE SPECIES, EXPOSED TO WINTER CONDITIONS

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Abstract. Recalcitrant seed-producing species occur even in temperate ecosystems. These species disperse seeds in autumn, while germination occurs in spring. “What proportion of seeds maintains viability over the winter?” was always a question. A research was conducted to evaluate the seed moisture content (SMC) and the viability of two recalcitrant and three orthodox species exposed to winter conditions. Seeds were collected from a forest near Baia-Mare, Cluj-Napoca, Romania and transported to the Department of Chemistry and Biology, North University Center of Baia-Mare. Experiments were initiated within 2 days. Seed coat ratio (SCR) was calculated to predict seed desiccation sensitivity. Seed germination was studied at 25ºC in light/dark (12 /12hr) conditions to evaluate viability. SMC of all the species was determined using the oven-dry method. According to the SCR test, probability of *Quercus rubra* and *Q. petraea* seeds to be recalcitrant was >0.5 confirming the recalcitrant storage behaviour. Further, SMC of these two species was >20% indicating that they are capable of maintaining high SMC even after exposure to winter conditions. Except for *Gleditsia triacanthos,* >50% of intact seeds of the studied species germinated, revealing that two recalcitrant seed-producing species had the ability to maintain viability even under winter conditions similar to the orthodox species tested. Scarified *G. triacanthos* seeds germinated >80% indicating that they have physical dormancy even they were exposed to winter conditions for 3 months. It can be concluded that a high proportion of recalcitrant seeds can survive after going through winter similar to orthodox seeds.

Keywords: desiccation sensitive; longevity; orthodox; recalcitrant.

INTRODUCTION

Seed storage behaviour type plays an important role in determining the longevity of seed under particular environmental conditions [10]. Recalcitrant seeds lose viability when they were dried < ~ 10 % seed moisture and have a short storage life [20]. Especially tropical recalcitrant seeds are also sensitive to the low-temperature conditions and lose viability under storage at temperatures < 10°C [10]. In contrast, orthodox seeds have a long storage life and tolerate desiccation. Recalcitrant storage behaviour type has been identified as an adaptation to establish seedlings as soon as they disperse [4]. Thus, recalcitrant seed-producing species are dominant in tropical aseasonal moist environments as these environments favor quick seedling establishment [19, 25]. Nevertheless, there are reports of species producing recalcitrant seeds even in temperate ecosystems where high seasonality in climate conditions is prevailed [13]. Most of the species producing recalcitrant seeds in the temperate zone are woody species and these species disperse seeds during the autumn and seed germination occur during the spring [13]. Thus, how these seeds maintain viability throughout the winter period was always a question. (1) Whether these seeds maintain seed moisture content high enough to maintain their viability during the winter and (2) what is the percentage of seeds survive after winter conditions? are the two initial questions to answer. Thus, research was designed to evaluate the seed moisture, viability and dormancy state of dispersed seeds of two recalcitrant and three orthodox species in Baia Mare, Romania. Tilki (2010) [24] has studied the effect of storage on germination and moisture content of *Q. petraea,* one of the species selected for our study. He found that storage time has not affected the moisture content of the seeds under laboratory moist storage conditions. However, the viability of the seeds has drastically reduced after storage for 17 months. However, change of viability or seed moisture content under natural conditions has not been studied.

MATERIALS AND METHODS

The studied species. Five common tree species found in native flora of northwestern Romania (town Baia Mare, 47.6567° N, 23.5850° E), were studied in this research. The species selected for this study are important in several ways. *Quercus petraea* is an edifying species for the regional forests in natural ecosystems. It is native to Europe occupies the hill area, and the forests have high biodiversity and provide habitat for important flora and fauna species. These forests are also, of great economic importance and forest management aims to produce high quality timber [15]. *Rosa canina* is also a native species which has great ecological amplitude. Together with other native shrub species, *Rosa canina* builds ecological corridors, which lead to reduce the fragmentation effect of forest habitats [17]. *Quercus rubra* [22], *Gleditsia triacanthos* [21] and *Catalpa bignonioides* [14] are exotic species to Romania as well as to Europe. These species are planted in urban spaces and in parks as ornamentals. Among them, *Quercus rubra* has been reported with invasive potential [22].

Seed collection. Seeds were collected from the ground below at least five randomly selected individuals from each of the five studied species from a forest patch in Baia-Mare, Romania in mid-February. All the seeds were dispersed in autumn and they were on the ground nearly for four months before they were collected. Seeds were put in polythene bags and transported to the Department of Chemistry and Biology, North University Center of Baia-Mare, Technical University of Cluj-Napoca, Romania. Experiments were initiated within two-days from seed collection. Until that seeds were maintained at room temperature.

Confirmation of seed desiccation sensitivity/ tolerance based on seed coat ratio data. Minimum of eight individual seeds was dissected into their component parts (endocarp / testa and embryo / endosperm). Seed coat ratio (SCR) was calculated using the following equation [5]:

$$\mathrm{SCR} = \frac{\text{dry weight of the covering structures (endocarp and testa)}}{\text{dry weight of the total dispersal unit}}$$

Following equation was used to predict the likelihood of desiccation sensitivity where a is SCR and b is $\log_{10}$ (seed dry weight) in grams.

$$P = \frac{e^{3.269 - 9.974a + 2.156b}}{1+e^{3.269 - 9.974a + 2.156b}}$$

If $P > 0.5$ seeds are likely to be desiccation sensitive. If $P < 0.5$ seeds are likely to be desiccation-tolerant. However, this experiment was not conducted for seeds of *Catalpa bignonioides* and *Rosa caninas* as they were very small.

Seed germination. Four replicates of seeds from each species were incubated on moistened tissue papers in Petri dishes at 25 °C in light/dark (12 hr/ 12 hr) conditions. Seeds were observed for germination at 2-day intervals for 30 days. Radicle emergence (about 0.5 mm) was the criterion for germination. The same experiment was conducted for the manually scarified seeds of *Gleditsia triacanthos* as intact seeds were not germinated. None germinated seeds were cut and embryos were observed to determine the viability (firm white viable embryo vs. soft dark non-viable embryo). Logistic 4 parameter sigmoidal curves were fitted to the germination data to determine the pattern of seed germination.

Seed moisture content (MC). Ten seeds from each species were weighed individually with a digital balance, oven-dried with forced convection at 120 °C for 3 hrs and reweighed. Seed moisture content was calculated on a fresh mass basis [11].

RESULTS

Confirmation of seed desiccation sensitivity/ tolerance based on seed coat ratio data. Probability of desiccation sensitivity of *Quercus rubra* and *Quercus petraea* was measured based on seed mass and seed coat ratio (SCR). According to the obtained results, P(D-S) of both species higher than 0.5 revealed that they are likely to be desiccation sensitive.

Seed germination. Over 50 % of the intact seeds of four of the five species germinated on distilled water without any treatment, whereas, none of the intact seeds of *G. triacanthos* germinated within 30 days of the study period (Fig. 1). However, when *G. triacanthos* seeds were manually scarified, they germinated > 80 % within a few days. Remaining seeds had a soft dark-coloured embryo indicating that they were none viable. Seeds of all the species had T_{50}< 30 days except for intact *G. triacanthos* seeds.

Seed moisture content (MC). Fresh seeds of two of the five species tested, *Q. rubra* and *Q. petraea* had > 20% MC whereas the seeds of rest of the study species had < 20% MC (Table 1).

DISCUSSION

Seed coat ratio test revealed that *Quercus rubra* and *Quercus petraea* seeds have a high possibility to be recalcitrant in storage behaviour. These two species have been reported as recalcitrant species by Pritchard (1991) [18, 24]. Further, Kew Millennium seed bank database suggested that these two species have recalcitrant storage behaviour. Although the seed coat ratio experiment was not conducted for *R. canina, G. triacanthos* and *C. bignonioides* seeds, they have been reported as desiccation tolerant [3, 8, 16]. Thus, these species provide a good opportunity to study whether recalcitrant seeds maintain their moisture condition during the winter and whether recalcitrant seeds maintain viability during the winter in a similar rate to desiccation tolerant seeds.

Table 1. Moisture content, germination % and dormancy of studied species

Species	Family	Origin	MC	Germination %	T_{50} (days)	Dormancy status at the time of the collection
Quercus rubra	Fagaceae	Alien invasive	26.23	87.5	5	ND
Quercus petraea	Fagaceae	Native	28.12	66.7	21	ND
Gleditsia triacanthos	Fabaceae	Alien	5.07	83.3	12	PY
Rosa canina	Rosaceae	Native	17.5	66.7	19	ND
Catalpa bignonioides	Bignoniaceae	Alien	15.6	50	24	ND

Table 2. Probability of desiccation sensitivity based on seed mass and seed coat ratio (SCR)

Species	Seed mass (g)	SCR	P (D-S)	DS/DT
Quercus rubra	4.46	0.321	0.811	DS
Quercus petraea	3.37	0.252	0.868	DS

DS- Desiccation sensitive, DT- desiccation tolerance

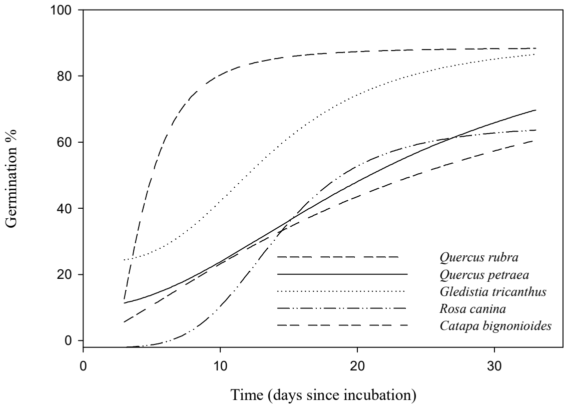

Figure 1. Cumulative germination of (A) *Quercus rubra*, (B) *Quercus petraea*, (C) *Gleditsia triacanthos*, (D) *Rosa canina* and (E) *Catalpa bignonioides* seeds incubated at 25 °C in light/dark (12 h/12 h) condition. Logistic 4 - parameter curve was fitted to raw data to determine T_{50}.

The seed moisture content of two recalcitrant species, *Q. rubra* and *Q. petraea* was slightly higher than 25 % which is within the range of recalcitrant species and thus, enough to maintain their viability. *R. canina* and *C. bignonioides* seeds have a higher moisture content compared to that of orthodox seeds [10]. This may be due to the high moisture content prevailed during the winter in the seed collected area. However, seed moisture content of *G. triacanthos* is very low compared to the other four species. This is because of the water-impermeable seed coat they have. Water impermeable seed coat with the hilum of Fabaceae seeds act as a water gauge controlling the moisture content of the seeds [12]. This seems to be allowing seeds to maintain low seed moisture content, improving the longevity of the seeds.

Eighty-seven per cent of the ground collected *Q. rubra* seeds germinated while the remaining seeds were non-viable. This revealed that *Q. rubra* seeds maintain high viability even after exposing winter conditions for > 3 months. *Q. petraea* seeds also germinated to > 60 % indicating that a considerable portion of seeds of this species retained viability even after exposing winter conditions. The proportion of *Q. petraea* seeds retaining viability is similar to that of the *R. canina* seeds while even higher than the proportion of viable seeds of *C. bignonioides*. This revealed that recalcitrant seeds have the capability to maintain seed viability under winter conditions similar to or sometimes even better than desiccation tolerant orthodox seeds. However, it would have better if this experiment is conducted till the end of the winter season where it would have allowed us to determine the true ability of these seeds in maintaining moisture condition and viability.

Fresh intact seeds of *R. canina* [1] have been reported to have physiological dormancy. However, the seeds exposed to winter conditions during the current experiment were non-dormant showing that winter conditions have alleviated seed dormancy of these two species. Susuzka and Bujarska-Borkowska (1987) [23] have also shown that seed dormancy of *R. canina* could be alleviated by 112 days of stratification under natural winter conditions. Although, no report of seed germination and dormancy of *C. bignonioides,* Francis (1990) [6] has reported that *C. longissima* seeds have no dormancy. Similarly, in our experiments, we observed that seeds of *C. bignonioides* exposed to winter conditions have no dormancy.

Intact *G. triacanthos* seeds have not germinated, while they also have not shown signs of imbibition indicating that seeds of this species have physical dormancy. Further, when these seeds were scarified (manually with a razor blade) they germinated to > 85 % and all the seeds enlarged showing that they imbibed after scarification. This further confirms the physical dormancy of the *G. triacanthos* seeds. Heit (1967) [9] and Geneve (2009) [7] have reported that seeds of *G. triacanthos* seeds have physical dormancy similar to other studied *Gladitsia* species [7] and similar to most of the other members of Fabaceae (as reviewed [2]). However, our observations reveal that exposure to winter conditions for 3 months have not alleviated physical dormancy of *G. triacanthos*. Further, exposure to winter conditions or exposure to winter and summer conditions may be required to break the physical dormancy of this species.

We can conclude that seeds of the two recalcitrant species - *Q. rubra* and *Q. petraea* - had the ability to retain moisture condition in a level needed for viability retention even after exposing to severe winter conditions. Further, it can be concluded that the tested two recalcitrant species have the ability to maintain the viability even after they were exposed to winter conditions for 3 months. Further, it was observed that the physical dormancy of *G. triacanthos* seed has not alleviated by exposing them to winter conditions for three months even though the physiological dormancy of *R. canina* and *C. bignonioides* is alleviated by the same conditions.

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