

## The Effect of Wintering on Secondary Seed Dispersal of Six Tree Species in a Zagrosian Forest Plantation

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**Abstract:** Continuous transplanting is necessary for sustained afforestation in arid environments. Yet the reproductive potential of the exotic trees planted in these areas has not been assessed. At the onset of winter, this study showed that except for *Ailanthus altissima*, seed viability of five other major trees (*Fraxinus angustifolia*, *Robinia pseudoacacia*, *Cercis siliquastrum*, *Quercus castaneifolia* and *Thuja orientalis*) planted in a Zagrosian site with a cold and dry climate, was higher than 60%. The results also showed that once these seeds were artificially placed on different secondary dispersal routes during winter, variation in number of lost seeds and viability percentage was high. Excluding *Quercus*, number of lost seeds was equal in surface scattered and buried seeds. The least mortality was observed in either soil buried or plastic covered seeds. The latest not only benefited from natural dormancy breaking conditions of overwintering, but also were protected from main above (birds) and under (insects) the soil surface predators.

**Key words:** Forest plantation • Natural regeneration • Seed viability

### INTRODUCTION

Forest plantations in arid and semi-arid areas are usually based on short and mid term afforestation projects and play an important role in alleviating pressure on neighboring land uses [1]. Earlier surveillances have shown that steppe-like regions in the low-rainfall areas of Southwest Asia are not naturally forested at their climax ecological status (e.g. [2]); hence artificial plantations have to be maintained under constant care and vigilance and a wide range of operations including irrigation, sanitation cutting and transplantation are currently underway in these plantations [3].

Despite high costs of the transplantation operations, natural regeneration potential of the exotic trees and endemic shrubs planted in these projects has not been estimated; seemingly because of an implicit assumption that the unfavorable weather conditions is a major obstacle to any successful natural regeneration establishment. Since seedling recruitment is greatly influenced by seed dispersal routes, to examine the feasibility of a natural regeneration strategy, this research was conducted to look at the post-dispersal fate of some tree seeds as the units of sexual reproduction.

Seed dispersal is a complex multistage phenomenon. It consists of seed removal from the mother tree, mainly as fruit, before landing on the ground or another objects (primary dispersal) and its consequent vertical and horizontal movements to a possible seedling establishment location (secondary dispersal) [4].

Mechanisms of secondary seed dispersal are poorly understood, yet previous studies have highlighted the great importance of the secondary stage of dispersal in seed fate, seedling recruitment and consequently the whole process of natural regeneration (reviewed partly by [5]). Coinciding the time of seed shedding of most wild woody plants in temperate regions with the period of changes in weather and animal behavior in late autumn, makes wintering a major cause of secondary seed dispersal. Several issues, including topographic and climatic variables as well as frugivory and seed predation by a broad range of biological factors [6], have been advanced to explain seed removal and loss at small spatial scales. Moreover, as seeds are scattered around, depend on the post-dispersal location and cover, not only their viability and vigor vary considerably, but also the levels of all kinds of seed dormancy may change throughout winter; which makes reaching to a conclusive prediction

about the in situ germination a challenging task. To address these issues, the current study also tried to compare the viability of the seeds at different destinations following release from parent plant in the region throughout winter.

## MATERIALS AND METHODS

**Study Site:** This study was carried out in Tahlijan forest plantation (Fig. 1) lying on the outskirts of the town of ShahreKord – Iran, at 2110 m asl, 32°20'15" N, 50°51'3" E, in fall and winter 2008-2009. The region has a semi-arid climate with dry summers and cold winters. The year of study was a normal year, with average precipitation of 321 mm, 125 days with subzero temperatures and stable snow cover. The local loamy clay soil surface is naturally dominated by annual grasses with scattered thorny shrubs and is currently planted with different tree species since the 1970s.

**Seed Sources:** A large amount of fruits were hand collected from six tree species with the highest cover in the region, namely: *Fraxinus angustifolia* Vahl., *Ailanthus altissima* (Mill.) Swingle, *Robinia pseudoacacia* L., *Cercis siliquastrum* L., *Quercus castaneifolia* C. A. Mey. and *Thuja orientalis* L.; which will be referred to as their scientific genus names hereafter. Seeds were extracted from *Robinia* and *Cercis* pods and *Thuja* cones for both lab and field studies and from the fruits of other three species only for viability tests. We refer to samara and acorn as seed here.

**Initial Viability Tests:** To evaluate the germination potential of the seed lots used in this study, 1) besides the control, a portion of *Robinia* and *Cercis* seeds underwent three different scarification treatments, which were boiling water until cooling down; mechanical abrasion using sand paper and concentrated sulfuric acid for one and 10 minutes, respectively (optimal acid treatment times based on previous pilot studies); 2) *Thuja*, *Ailanthus* and *Fraxinus* seeds were put between layers of wet sand in well ventilated conditions at 4°C for 0, 3, 5 and 6 weeks (cold stratification).

Seeds of these five species were incubated at 23°C on two layers of filter paper in glass Petri dishes of 50 seeds with four replications in each treatment. Arcsin square root transformed germination percentages were analyzed using One-Way ANOVA, followed by a Tukey's

test ( $P = 0.05$ ) to determine differences among treatment means. Fifty *Quercus* seeds were randomly selected and their viability was tested by tetrazolium chloride staining test.

**Seed Dispersal Treatments:** In the fourth week of November, based on a nested design, another portion of the above mentioned seed collection were placed on separate seed beds in different parts of a well protected two-hectare plantation area that had been already divided into quarters (Fig. 1). The randomized systematically selected main plots (including the mimic of all possible natural secondary seed dispersal routes), consisted of: 1- scattering the seeds over 1.5×1.5 square meters on the soil surface, 2- shallow burial of the seeds in the soil, 3- covering the seeds of the first treatment with a camouflage opaque plastic sheet, 4- placing the seeds into loosely capped crystal jars and 5- placing the seeds into a soil-proof stainless wire mesh-cage (Fig. 2). Using a small dark brown stain of acetone based formaldehyde-free nail polish, seeds in the three first treatments were tagged on each side. Care was taken not to inhibit the possible radicle growth.

Since the plantation was irrigated using parallel furrows spaced at regular intervals of 4 meters, different seed species might show significant differences in viability and dispersal pattern inside and outside the furrows. Therefore two subplots were set out. On the first subplot we considered micro topographic positions (inside and outside the furrows) and the six seed species were our choice for the second subplot. The experiment was run in four replications for main plot and subplot of species and two replications of topographic position in each quarter and in total 16, 16 and 8 replications for each source of variation, respectively.

**Final Viability Tests:** After removal a few broken pots and torn plastic sheets, seeds of each sample were gathered separately in the second week of March. The number of remaining tagged seeds counted over a 3×3 square meters area. Viability tests were then conducted on all the recovered seeds in the same way previously described for the pre-scattered seeds. Unlike the initial tests, however, no dormancy-breaking treatment was applied and the nested ANOVA was run for each of the total germination values, as well as the number of lost tagged seeds separately. The general linear model (GLM) function in Minitab 15 was used to compute all the analyses and comparisons.

## RESULTS

**Viability Tests:** The results indicated a high viability rate ( $83\% \pm \text{SD. } 7.4$ ) for *Quercus* seeds, tested by tetrazolium. In the case of other species, analyses of variance on the seed dormancy breaking results, revealed highly significant differences ( $P < 0.01$ ) among both scarification and stratification treatments (ANOVA tables are not presented).

Compared with *Robinia* seeds abraded with sandpaper, boiling water and sulfuric acid had more significant effects on the rate of dormancy release. More than two thirds of these seeds germinated. These treatments resulted similar germination for *Cercis* seeds. By comparison, *Cercis* seeds treated with acid gave statistically better germination results (Fig. 3).

From a statistical point of view, the outcome of stratification treatments showed that the *Thuja* seed lot was not physiologically dormant. As they underwent

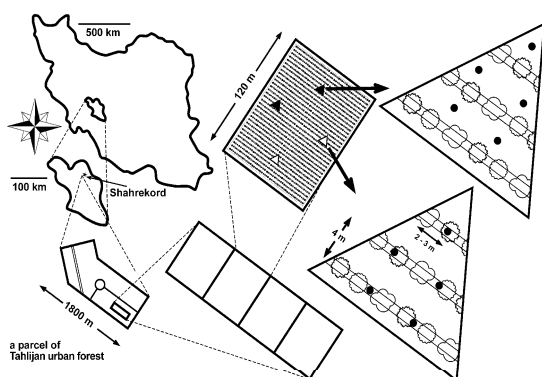


Fig. 1: Study site of the seed dispersal experiment, in Tahligan plantation near Shahrekord, the capital of Chaharmahal-va-Bakhtiari province. In each densely planted quarter of the site, black and white triangles represent the collection of five different seed beds (black circles) placed respectively outside and inside the irrigation furrows.

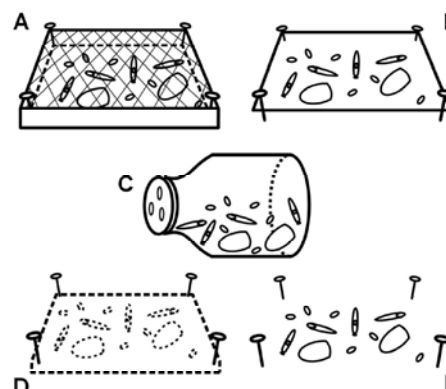


Fig. 2: Five different seed beds used as main plot in the study: A. soil-proof stainless wire mesh-cage; B. camouflage opaque plastic sheet cover; C. loosely capped crystal jar, D. shallow soil burial and E. scattering on the soil surface. The treatments of B, D and E are also considered as the secondary dispersal routes.

different stratification periods, their germination percentage did not change and remained constantly high. *Fraxinus* seeds, on the other hand, positively responded to the treatments. The longer stratification period, in this species, the greater dormancy breakage rate. In contrast to relatively high germination in *Fraxinus*, even six weeks of cold stratification did not increase germination rate of *Ailanthus* seeds to more than eight percent in average (Fig. 4).

**Seed Fate During Secondary Dispersal:** The results showed significant differences among dispersal locations and species of the seeds, however, the subplot of the micro topographic position had no meaningful effect on the results (Table 1). *Quercus* seeds exhibited different spatial dispersal pattern. Almost all the acorns scattered on the soil surface disappeared, which was considerably higher than other two dispersal routes

Table 1: Analysis of variations caused by wintering on the lost-seed and viability percentages (Arcsin square root transformed) based on a nested ANOVA, seed dispersal location as main plot and micro topographic position along with species as subplots

Measured variable	Source of variation	Degree of freedom	Mean Square	F statistic
Number of lost-seeds	Main plot (dispersal locations)	2	5497	33.13**
	Micro topographic position	3	165.9	0.21 <sup>n.s.</sup>
	Species	30	791.4	9.89**
	Error	180		
Seed viability	Main plot (dispersal locations)	4	9923.9	27.21**
	Micro topographic position	5	364.7	0.33 <sup>n.s.</sup>
	Species	50	1096.1	7.75**
	Error	300	141.4	

n.s.: not significant; \*\*:  $P < 0.01$

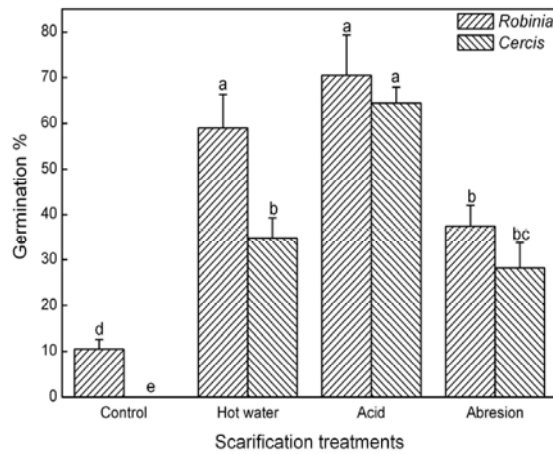


Fig. 3: The effect of different scarification pretreatments on seed germination of two hard seed species before dispersing to the objective site. The vertical bars represent the standard deviation. Means with the same alphabets do not differ significantly from each other.

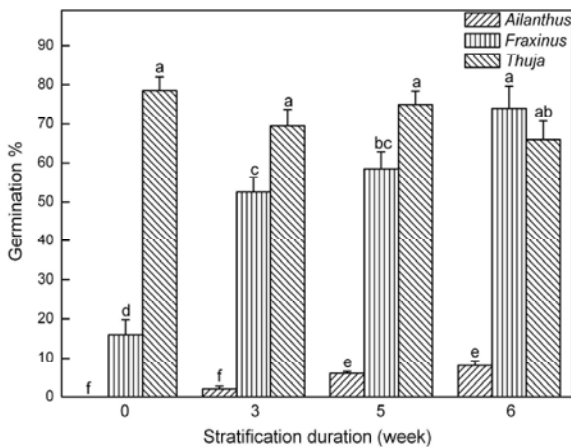


Fig. 4: The effect of different stratification durations on germination of three seedlots with presumed physiological dormancy, before dispersing to the objective site. The vertical bars represent the standard deviation. Means with the same alphabets do not differ significantly from each other.

and also other species. More than half of the surface-scattered *Ailanthus* seeds, for instance, were recovered after wintering. Lost seeds of other species were mainly among both the surface-scattered and buried seeds. In *Cercis*, soil burial was the primary reason for seed lost. In general plastic sheet

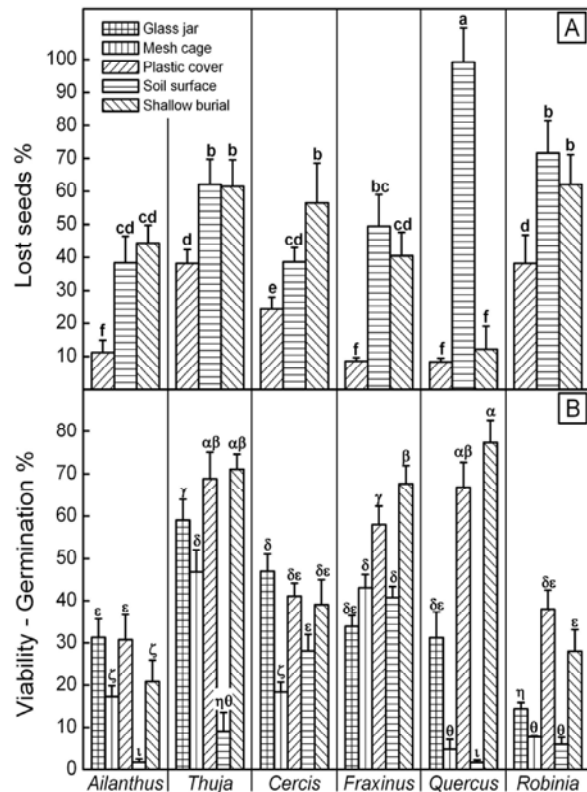


Fig. 5: The effect of wintering on the percentage of viability (B) and number of lost seeds (A) of five seedlots, located on different seed beds. The vertical bars represent the standard deviation. Means with the same Roman alphabets (percent of lost seeds) and Greek alphabets (viability percentage).

preserved seeds significantly better than the other two dispersal routes and diminished the number of lost seeds to approximately 10 percent in *Quercus*, *Fraxinus* and *Ailanthus* (Fig. 5).

Comparing with the initial viability values; wintering adversely affected germination of the surface-scattered seeds. Next to this treatment, mesh-caged seeds exhibited the lowest viability. Excluding *Ailanthus*, the shallow burial of the seeds followed by the plastic cover treatment were the dispersal treatments that maintained the highest level of viability. In general, seeds of *Quercus*, *Thuja* and *Fraxinus* recovered from different dispersal treatments, retained higher levels of viability (about 60-80%). Seeds of other three species, on the other hand, showed an inconsistent decrease in germination percentage (Fig. 5).

## DISCUSSION

By considering both the secondary dispersal locations (seed dispersal routes) and species of the seeds, wintering caused significant differences in germination potential and number of lost seeds (Table 1). Based on the assumption that most seeds released from mother trees would be predominantly ended up on the soil surface, the results showed diminishing consequences of wintering on the seed viability in an East Zagrosian exotic plantation. However post-dispersal seed fate was significantly affected by the place on which seeds were located.

The mortality was considerably decreased when seeds were covered with a layer of shelter, indicating the significance of exposing to air [7] on senescence acceleration and seed aging. Extreme low temperatures may cause imbibitional chilling injury [8]; or increase seed sensitivity to environmental stresses and raise the speed of seed deterioration process [8]. Minimum daily temperatures of less than -10°C are prevailing during winter in the region and an absolute minimum of -17°C was recorded in the year of study. Drastic changes in moisture content [10] and high light intensity [11], may also be regarded as limiting factors to uncovered seeds. The latest can particularly be considered for *Thuja*, *Fraxinus*, *Quercus* and *Robinia*, since significant differences were observed between viability of radiation unprotected (crystal jar incubated) and protected (plastic sheet covered as well as soil buried) seeds of these four species.

Frequent drying in two scattering treatments (surface-scattering and mesh-caging) can be the cause of viability deterioration in recalcitrant *Quercus* seeds [12]. On the other hand, when considering better protected treatments (plastic sheet cover, crystal jar and soil burying), alleviation of physiological dormancy in *Fraxinus* and *Ailanthus* seeds, may be attributed to preservation of seed moisture content (the common characteristics of these treatments) during cold season. Altering temperature on the soil surface can also accelerate the dormancy release [13].

Contrary to current seed manuals [14], increase of dormancy breakdown rate after wintering in *Ailanthus* seeds may suggest that more than two months of stratification was needed to overcome physiological dormancy in this species, which is consistent with Djazirehi [15].

Choosing among surface scattered orthodox seeds, *Cercis* and *Fraxinus* showed the highest germination percentage after wintering, probably because these

species were the only ones originated from close by natural forest site. In case of *Cercis*, although we considered seed coat-imposed dormancy as the main factor for germination delay [16], but the over wintering results confirm the existence of a shallow physiological seed dormancy [17] in this species.

The effect of on-the-ground entomological and microbial activities does not appear to be significant for *Robinia* and *Fraxinus*, since viability decrease was not different between surface-scattered and mesh-caged seeds. However, difference between the two treatments was more distinct for seeds of other four species, strengthening the importance of biological deterioration on the fate of these seeds [18, 19].

The projected size of the seeds of *Quercus* dismisses the possibility of wind or water transportation and predation by vertebrates is usually accounted for secondary seed dispersal in oaks [20]. Wild boar and nine rodent species are common mammals in the region, which may readily consume the acorns [21]. However, the major difference in number of lost acorns in surface scattered and plastic sheet covered treatments indicates the significance of the predation by birds, which use vision rather than olfaction to perceive seed locations [22, 23]. Magpie and two crow species are among non-immigrant birds in the region, which have large enough bills to feed on the acorns [24].

Apart from *Quercus* and *Cercis*, the same number of lost seeds in surface-scattering and soil burial treatments of other species indicates the significance of soil-dwelling organisms, primarily ants [25] in dispersal of small and winged seeds. As a result, notably low portion of lost seeds in plastic covered treatment may not only indicate the importance of wind and birds in removing the seeds, but also may highlight the avoidance of seed-eating insects from reaching to soil surface during winter.

## CONCLUSION

As far as we know, no other study has been conducted examining the seed fate in forest plantation areas using artificial seed beds. According to our results, there are good reasons (effects of wintering as well as predating activity of soil organisms) to explain the failure of seed planting projects in nearby forest and poor natural regeneration in these plantations. While the variation in spatial secondary seed dispersal is immense, the study did show that covering the seeds with a layer of shelter can significantly decrease the chance of degradation, predation and micro transportation in the plantation site.

Phenology data collected from student projects have shown that except for *Quercus* and the evergreen *Thuja*, time of commencement of seed (fruit) shedding is, in average, more than two weeks after leaf senescence date. Based on these results, therefore, our suggestion for a successful natural regeneration and seedling recruitment is collecting the seeds and dispersing them before leaf fall in November. At the end, the results showed that among the five species examined, Seeds of *Ailanthus* are the least compatible for sexual reproduction in the region. In fact, natural regeneration of this species is generally attributed to root suckers.

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