forests

Article

Dormancy-Breaking and Germination Requirements for Seeds of *Sorbus alnifolia* (Siebold & Zucc.) K.Koch (Rosaceae), a Mesic Forest Tree with High Ornamental Potential

Yuhan Tang, Keliang Zhang, Yin Zhang and Jun Tao *

Jiangsu Key Laboratory of Crop Genetics and Physiology, College of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, China; DX120180105@yzu.edu.cn (Y.T.); zhangkeliang@yzu.edu.cn (K.Z.); mx120180605@yzu.edu.cn (Y.Z.)

* Correspondence: taojun@yzu.edu.cn; Tel.: +86-0514-8799-7219

Received: 8 March 2019; Accepted: 4 April 2019; Published: 8 April 2019

Abstract: *Sorbus alnifolia* (Siebold & Zucc.) K.Koch (Rosaceae) is an economically important tree in the temperate forests of Eastern China. In recent decades, ever-increasing use and modification of forestlands have resulted in major degeneration of the natural habitat of *S. alnifolia*. Moreover, *S. alnifolia* seeds germinate in a complicated way, leading to a high cost of propagation. The current study aimed to determine the requirements for breaking seed dormancy and for germination as well as to characterize the type of seed dormancy present in this species. Moreover, the roles of temperature, cold/warm stratification, and gibberellic acid (GA_3) in breaking dormancy were tested combined with a study of the soil seed bank. The results showed that intact seeds of *S. alnifolia* were dormant, requiring 150 days of cold stratification to achieve the maximum germination percentage at 5/15 °C. Exposure of the seeds to ranges of temperatures at 15/25 °C and 20/30 °C resulted in secondary dormancy. Scarifying seed coat and partial removal of the cotyledon promoted germination. Compared with long-term cold stratification, one month of warm stratification plus cold stratification was superior in breaking dormancy. Application of GA_3 did not break the dormancy during two months of incubation. Seeds of *S. alnifolia* formed a transient seed bank. The viability of freshly matured *S. alnifolia* seeds was 87.65% ± 11.67%, but this declined to 38.25% after 6-months of storage at room temperature. Seeds of *S. alnifolia* have a deep physiological dormancy; cold stratification will be useful in propagating this species. The long chilling requirements of *S. alnifolia* seeds would avoid seedling death in winter.

Keywords: cold stratification; deep physiological dormancy; seed dormancy; seed germination; soil seed bank; *Sorbus alnifolia*

1. Introduction

Seeds of temperate forest plants may possess various morphological and physiological characteristics that partially represent the plant's response to selective pressure within the current environment or previous environments during plant evolution [1,2]. As a typical feature of seeds, seed dormancy can prevent seeds from germinating in the presence of inappropriate environmental conditions, allowing more seedlings to survive over time [3–5]. Typically, seed dormancy contributes to screening the best time and place for seed germination; thus, seed germination data are critical for gaining an understanding of community processes, such as the establishment and succession of plants, as well as natural regeneration [4–6]. On this account, seed dormancy can serve as a fundamental tool to manage the forest population [7]. Although a considerable amount of information is available on seed dormancy among the deciduous forest species in Eastern North America, information on seed dormancy and the dormancy mechanisms of specific species in this vegetation type in China is still scarce and dispersed [4].

Baskin and Baskin [4] have put forward five dormancy classes as part of a detailed system used to classify seed dormancy as follows: Physiological dormancy (PD), morphological dormancy (MD), physical dormancy (PY), morphophysiological dormancy (MPD), and combinational dormancy (CD). Moreover, they have further subdivided dormancy classes into levels and types where appropriate. Endogenous, as well as exogenous parameters, may assist in maintaining or releasing dormancy [4,8], but the embryonic morphology, water permeability of the seed coat, and germinating ability among fresh seeds within one month of reaching maturity are the keys to feasibly determining the dormancy state [4].

Sorbus alnifolia (Siebold & Zucc.) K.Koch (Rosaceae), a frequently encountered forest plant observed within the mountainous areas in China, can grow to a height of 20 m. The simple, alternately arranged leaves have a serrated or lobed margin. It can produce white compound corymbs of flowers in April and May, which form red fruits in August and September; therefore, it is an important tree planted in parks, gardens, and wildlife areas [9]. Meanwhile, it grows extensively in slopes, gullies, mixed forests, and thickets of China, Japan, and Korea. In China, *S. alnifolia* occurs from Northeast to Southeast China, ranging from Heilongjiang to Fujian provinces. The dense wood of this species can be used for carving and turning; in addition, it can also be used to make tool handles and walking sticks [10]. The soft and juicy fruit is used for making jam, marmalade, various drinks, wine, and vinegar. Further, its flowers make this tree one of the most aesthetically pleasing, which is undoubtedly one of the additional reasons why it is frequently cultivated by humans. However, most natural and semi-natural forests that have *S. alnifolia* populations are declining, which can be attributed to anthropogenic disturbance; therefore, restoration efforts are required in numerous countries worldwide [11].

Seed propagation serves as a crucial approach to the cultivation of *Sorbus* species and is necessary for the breeding of novel cultivars [12]. However, the seeds of *S. alnifolia* experience deep dormancy levels at dispersal, which complicates seedling cultivation. Seed germination of *Sorbus* species usually occurs after several months in natural conditions, while the seedlings have a low survival rate. According to Devillez et al. [13], dormancy of *S. aucuparia* mainly originated from the embryo, while that of *S. aria* were subject to the predominant influence of the seed coat. Dormancy of *S. torminalis* had been recognized as the intermediate type. Most seeds of *S. commixta* and *S. aucuparia* cannot germinate without being first ingested by birds [14,15]. Oster et al. [16] examined the germination inhibitors within the fruits and seeds of *S. aucuparia*, finding three distinct inhibitors, including parasorbic, abscisic, isopropyl-malic acids. Although germination characteristics have been extensively studied in a number of closely related species [17], seeds of *S. alnifolia* are associated with dismal and fruitless germination attempts at plant nurseries, which may be related to inadequate artificial preconditioning of seed to break dormancy prior to sowing. Nevertheless, a comprehensive study related to the requirements of breaking dormancy and seed germination of *S. alnifolia* seeds under natural and simulated natural conditions has not been done.

This study mainly aimed to examine the conditions needed to break dormancy as well as those needed for seed germination, and to classify the type and depth of seed dormancy in *S. alnifolia*. Specifically, we investigated the effects of (1) scarification on imbibition to test for physical dormancy (PY), (2) temperature as well as light demands during germination, (3) both cold and warm stratification on breaking dormancy, (4) GA_3 on breaking dormancy as well as on germination, and (5) soil seed bank dynamics.

2. Materials and Methods

2.1. Seed Collection and Study Area

During September 8–14, 2017, freshly matured fruits of *S. alnifolia* were obtained from a natural population in Jinan, Shandong Province, China (36°57′99″ N, 116°79′61″ E, 67 m a.s.l.). The pericarps of the fruits were manually removed by rubbing them with hands. The seeds were stored at −18 °C after natural air-drying. To prevent a change in the physiological state of the seeds, experiments on seed germination had been started within 2 weeks following seed acquisition. The area of seed collection experiences a typical continental monsoon climate featuring a hot, rainy summer and cold, dry winter [18]. The average annual precipitation was 669 mm, among which, approximately 70% falls from June to August; meanwhile, the annual average temperature was 13.8 °C, with that in the coldest (January) and hottest (July) month being −0.2 °C and 27.2 °C, respectively.

2.2. Seed Morphology and Size

The length, width, and thickness of 20 seeds were determined using a Vernier caliper (Santo 8041, Santo Corp., Shanghai, China). Ten randomly-chosen replicates of 100 fresh seeds were weighed using an electronic balance having precision to 0.0001 g (BP 221, Sartorius, Inc., Goettingen, Germany) to determine mass. Seed viability of freshly matured seeds and seed checked after 6 months of storage under ambient temperatures (at 20 °C–25 °C and relative humidity of 40%–50%) was tested by triphenyl tetrazolium chloride (TTC).

2.3. Water Imbibition Experiment

Water-imbibition experiments were carried out in the laboratory (20 °C–25 °C, RH 40%–50%). Water uptake was compared between scarified and non-scarified seeds to detect the water permeability of the seed coats. Seeds were subject to scratching using a scalpel (a 1 mm cut opposite to the hilum); four duplicates for 25 respective scarified as well as non-scarified seeds were used. The weight of every duplicate was measured by an analytical balance; later, the duplicates were placed onto Whatman No. 1 filter paper moistened with distilled water in 10 cm diameter plastic Petri dishes. Seeds were removed from the Petri dishes at 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, and 72 h for measurement; the seeds were blotted dry, weighed, and replaced onto the wet filter paper. Subsequently, the percent increase of fresh mass (% W_r) was computed according to the formula $W_r = ((W_f - W_i)/W_i) \times 100$, where W_i represented the initial mass of seeds, and W_f was the seed mass following water absorption for a certain period of time [19].

2.4. Effects of Light and Temperature on Germination

Fresh seeds were tested for germination by incubating seeds at 5/15, 10/20, 15/25 and 20/30 °C (12/12 h) in light (12 h photoperiod/day, approximately 100 µmol $m^{-2}s^{-1}$, cool white fluorescent light; hereafter light) and in continuous darkness (seeds in opaque bags). Four replicates of 25 seeds each placed in 10-cm-diameter Petri dishes on two layers of Whatman No. 1 filter paper moistened with 5 mL distilled water were used for each treatment. All Petri dishes were then sealed by Parafilm^{TM}. For seeds incubated in light, the higher and lower 12 h daily temperatures were used to correspond to 12 h periods of light and dark, respectively. This alternating temperature scheme represented the air temperatures close to the average daily maximum and minimum in every month within its natural habitat in Jinan: 5/15 °C, April and October; 10/20 °C, May and September; 15/25 °C June and August; 20/30 °C, July. Germination numbers under light conditions were checked weekly for 60 days. Seeds subject to dark incubation were examined only after 60 days of incubation. The criterion for germination was radicle tip emergence $\geq$ 1 mm. Finally, the un-germinated seeds were pinched using a forceps to determine if they were viable based on whether they had a grey and soft embryo (non-viable) or a white and firm embryo (viable) [4].

2.5. Effects of Scarification and Partial Removal of the Cotyledon on Germination

To determine the effect of the seed coat and cotyledon on germination, four replicates of 25 seeds were selected for each of three treatments: (1) Untreated intact seeds, (2) scarified seed coat (a 1 mm cut opposite to the hilum), and (3) 1/2 of the cotyledon removed. Each set of 25 seeds were placed onto two layers of Whatman No. 1 filter paper moistened with distilled water in 10 cm diameter plastic Petri dishes, followed by 60 days of incubation at 5/15 °C, 10/20 °C, 15/25 °C, and 20/30 °C under light conditions. Germination numbers were examined at the end of the experiment.

2.6. Effects of GA_3 Treatment on Germination

To determine the effect of gibberellic acid (GA_3) on dormancy breaking, four replicates of 25 fresh seeds were incubated in 0 (distill water) 10, 100, or 1000 $mg \cdot L^{-1}$ of GA_3 (Sigma-Aldrich, St. Louis, MO, USA) solutions at 5/15 °C, 10/20 °C, 15/25 °C, and 20/30 °C under light conditions for 60 d. Subsequently, seeds were checked every week for germination. Meanwhile, water was added into the dishes when necessary.

2.7. Effects of Cold Stratification on Germination

Fresh seeds were placed evenly spaced onto two-layer filter paper and covered with washed quartz sands (5 cm in depth, 11%–14% water content in the sand) in a 20 cm diameter × 10 cm deep metal boxes, so as to examine the response of *S. alnifolia* seed to cold stratification. Next, the metal boxes were sealed and put into a 5 °C refrigerator for 30, 60, 90, or 150 days. Following each stratification period, the seeds were subjected to 60 days of incubation at 5/15 °C, 10/20 °C, 15/25 °C, and 20/30 °C under light conditions. Germination numbers were checked at the end of the experiment.

2.8. Effects of Warm Stratification and Warm + Cold Stratification in Breaking Dormancy

Because *S. alnifolia* seeds are dispersed during August and September, in the wild, they could experience two months of warm stratification in autumn before cold stratification. To examine the role of warm stratification as well as warm + cold stratification in germination, four replications of 25 fresh seeds each were incubated under two temperature regimes: (1) 20 °C for 1 and 2 months, then germinated at 5/15 °C for 30 days; (2) 20 °C for 1 and 2 months preceding to 2 °C for 5 months, then germinated in 5/15 °C for 60 days. Germination numbers were checked at the end of the experiment.

2.9. Dynamics of the Soil Seed Bank

Soil seed bank samples were collected from *S. alnifolia* habitat on August 1, 2017 (prior to seed dispersal), October 1, 2017 (following seed dispersal), April 1, 2018 (prior to seed germination), and June 1, 2018 (following seed germination). Within 2 m of the chosen *S. alnifolia* sample canopy, samples were collected in a 10 × 10 cm (length × width) area at a depth of 0, 0–2 and 2–5 cm soil for a total of 60 samples. The samples were put into a 1-mm soil sieve, followed by washing with tap water until only *S. alnifolia* seeds, gravel, and plant litter were present within the sieve. *Sorbus alnifolia* seeds in soil sample were picked out by hand. Meanwhile, the number of seeds was calculated.

2.10. Data Analysis

The germination numbers were converted into percentages based on the viable seed number; meanwhile, the percentage results were subjected to arcsine transformation prior to statistical analyses, so as to guarantee the homogeneity of variance. T-tests were used for two different treatments ($p < 0.05$), one-way analysis of variance (ANOVA) was used to test at least three treatments using one factor, and two- or three-way ANOVA was employed to test for more than two factors ($p < 0.05$). If an ANOVA indicated significant differences in the data, the differences among treatments were determined using a Tukey's HSD test ($p < 0.05$). All analyses were completed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software.

3. Results

3.1. Seed Morphology and Size

Seeds of *S. alnifolia* are brownish and linear-oblong shaped at maturity, with a length of 0.68 ± 0.04 cm, a width of 0.32 ± 0.02 cm, and thickness of 0.23 ± 0.01 cm. The thousand seed mass was 2.92 ± 0.37 g. The viability of freshly matured *S. alnifolia* seeds was 87.65 ± 11.67%, but this would decline to 38.25% after 6-months of dry storage at room temperature (20 °C–25 °C and RH of 40%–50%).

3.2. Imbibition of water

Both intact seeds and seeds with a scarified seed coat can imbibe water ($F = 188.49$, $p < 0.001$) (Figure 1), while seed coat scarification led to a slightly elevated water absorption rate. After imbibing water for 12 h, the seed mass of scarified seed increased to 70.08%, while the increase was only 56.01% for intact seeds. Water uptake peaked after 24 h, but the increase of the water absorption of the intact seeds was still significant.

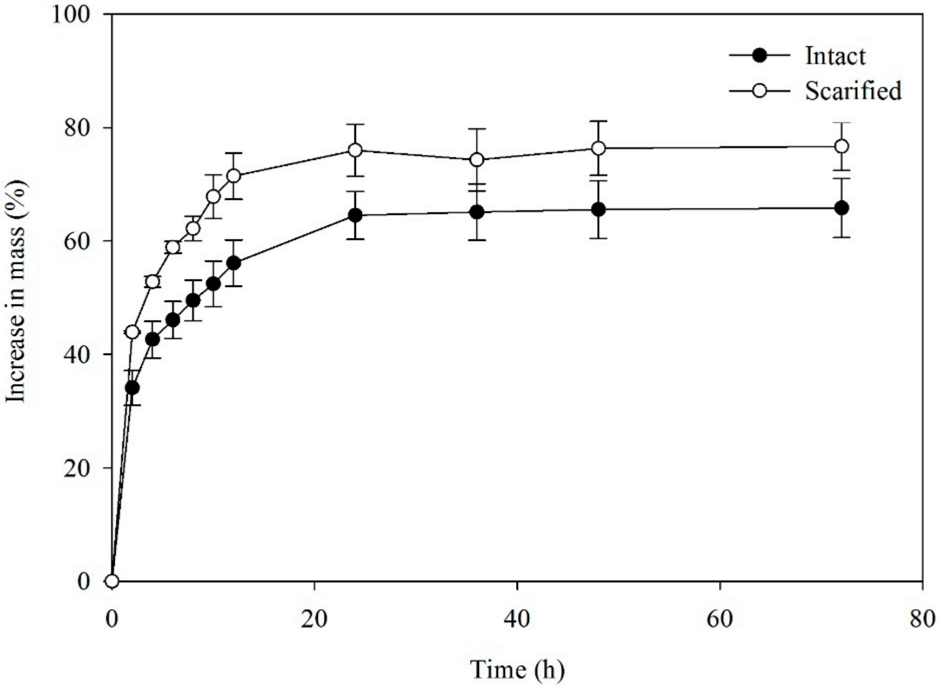

Figure 1. Imbibition curves for intact and scarified *Sorbus alnifolia* (Siebold & Zucc.) K.Koch seeds. Error bars are mean ± se.

3.3. Effects of Light and Temperature on Germination

No fresh seeds would germinate after incubation at 5/15 °C, 10/20 °C, 15/25 °C, and 20/30 °C under light/ dark conditions for 60 days.

3.4. Effects of Scarification and Partial Removal of the Cotyledon on Germination

Two-way ANOVA indicated that germination temperature ($F = 11.626$, $p < 0.001$), scarified seed coat or removal of 1/2 cotyledon ($F = 12.877$, $p < 0.001$) and their interaction ($F = 4.672$, $p = 0.001$) had a signification effect on the germination of *S. alnifolia* seeds (Figure 2). After 60 days of incubation, 20% of seeds with 1/2 the cotyledon removed germinated at 5/15 °C, whereas only 8% of seeds with scarified seed coat and no intact seeds germinated at 5/15 °C.

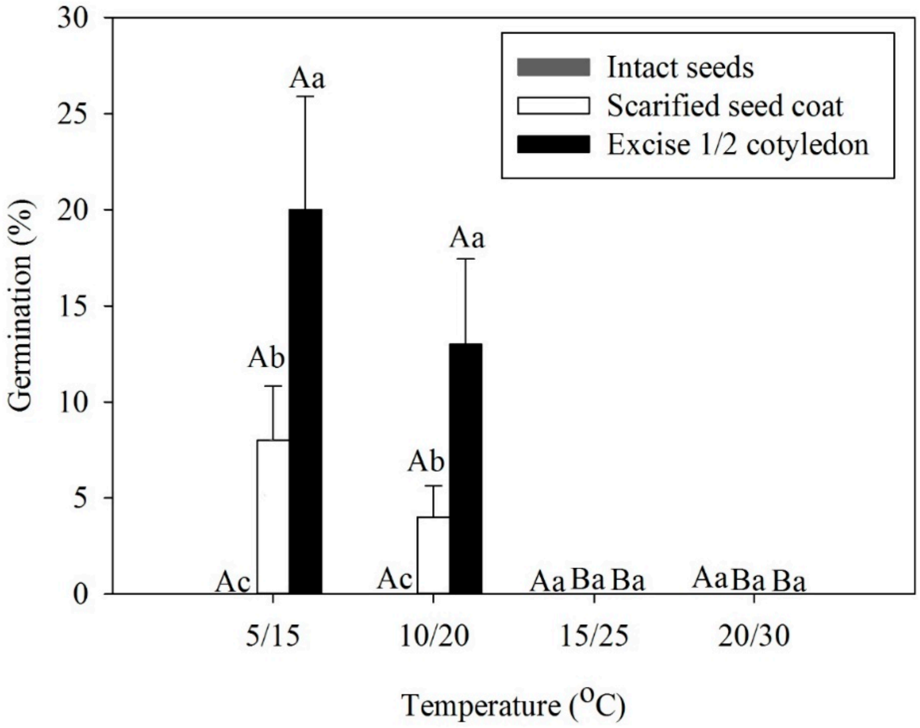

Figure 2. Effects of scarification and partial removal of the cotyledon on germination of *Sorbus alnifolia* (Siebold & Zucc.) K.Koch seeds. Error bars are mean ± se. Different uppercase letters indicate significant differences among temperatures in the same treatment, and different lowercase letters indicate significant differences among seeds of intact, scarified seed coat, and seeds with half of cotyledon excised at the same temperature ($p < 0.05$).

3.5. Effects of GA_3 Treatment on Germination

Sorbus alnifolia seeds treated with GA_3 did not germinate within 60 days; during that time no obvious change in the structure and hardness of the seed was observed, but mildew obviously grew on the seeds.

3.6. Effects of Cold Stratification on Germination

Results of two-way ANOVA indicated that germination temperature ($F = 387.121$, $p < 0.001$), duration of cold stratification ($F = 346.261$, $p < 0.001$), and the interaction between them ($F = 86.273$, $p < 0.001$) significantly affected the germination of *S. alnifolia* seeds (Figure 3). When seeds were subject to cold stratification for 30 days, no seeds germinated in 5/15 °C, 10/20 °C, 15/25 °C, and 20/30 °C. After cold stratification for 60 days, seeds would germinate to 22% and 5% at 5/15 °C and 10/20 °C (Figure 3), respectively. After cold stratification for 150 days, seeds would germinate to 91%, 48%, 13%, and 8% at 5/15 °C, 10/20 °C, 15/25 °C, and 20/30 °C, respectively.

3.7. Effects of Warm Stratification and Warm + Cold Stratification in Breaking Dormancy

Warm stratification resulted in a significant increase in germination of *S. alnifolia* seeds ($F = 19.000$, $p = 0.001$). After two months of warm stratification, >20% of seeds germinated. Cold stratification after warm stratification also significantly affected the germination of *S. alnifolia* seeds ($F = 7.539$, $p = 0.012$). After one month of warm stratification plus five months of cold stratification. >90% seeds germinated, but two months of warm stratification plus five months of cold stratification decreased germination to 70% (Figure 4).

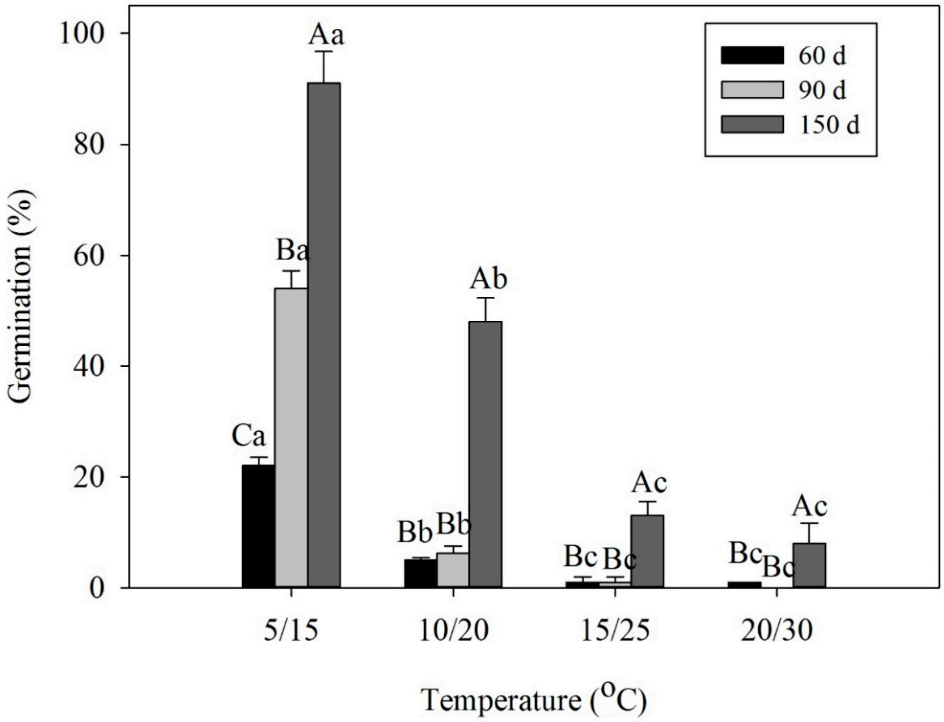

Figure 3. Effects of cold stratification on germination of *Sorbus alnifolia* (Siebold & Zucc.) K.Koch seeds. Error bars are mean ± se. Different uppercase letters indicate significant differences among temperatures at the same cold stratification time, and different lowercase letters indicate significant differences among different cold stratification times at the same temperature ($p < 0.05$).

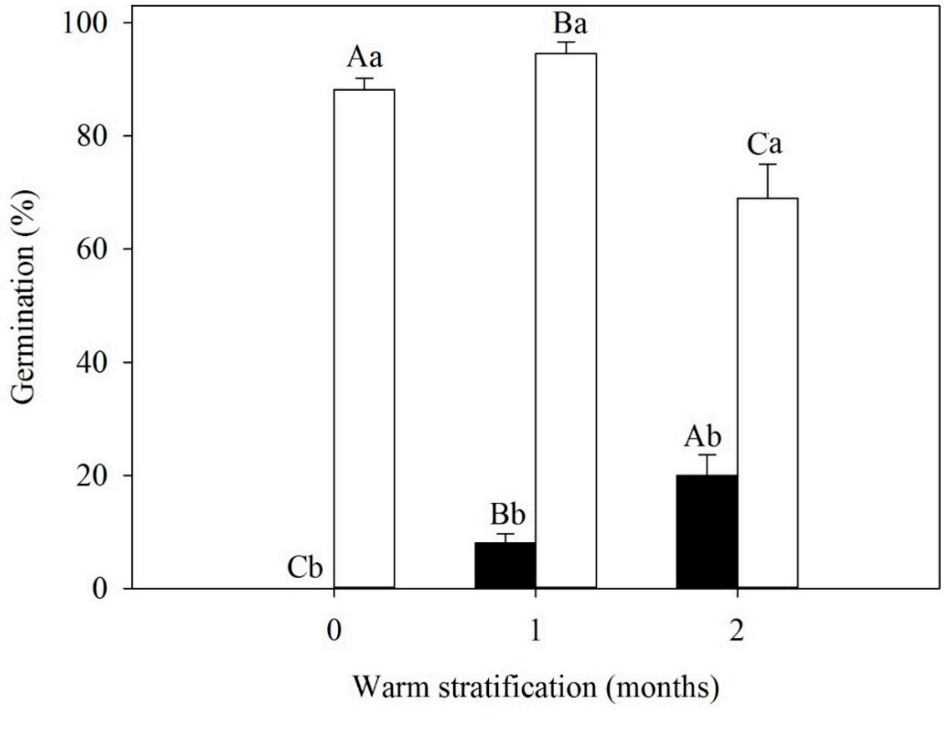

Figure 4. Effects of warm stratification (black bar) and warm + 5 months of cold stratification (white bar) on germination of *Sorbus alnifolia* (Siebold & Zucc.) K.Koch seeds. Error bars are mean ± se. Different uppercase letters indicate significant differences among different warm stratification time at the same stratification treatment, and different lowercase letters indicate significant differences between warm stratification and warm + 5 month of cold stratification at the same warm stratification time ($p < 0.05$).

3.8. Dynamics of the Soil Seed Bank

Season ($F = 9.227$, $p < 0.001$), depth of seeds ($F = 8.591$, $p < 0.001$), and the interaction between them ($F = 3.920$, $p = 0.001$) remarkably affected the seed in soil seed bank (Figure 5). The seed number on the surface of soil was evidently elevated compared with that in other soil depths. No seeds were in the seed bank in August before seed dispersal, and 303 ± 87 seeds were found in October after dispersal; this number decreased to 99 ± 57 seeds m^{-2} in April or to 21 ± 21 seeds m^{-2} after germination in June.

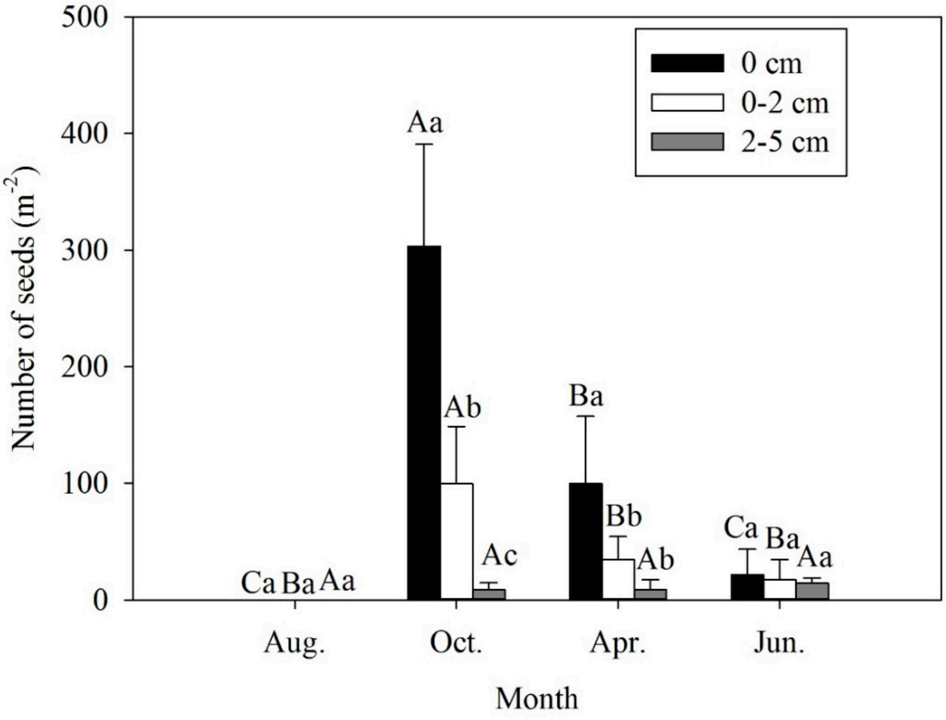

Figure 5. Dynamics of the soil seed bank of *Sorbus alnifolia* (Siebold & Zucc.) K.Koch in a natural habitat in 2017–2018. Error bars are mean ± se. Different uppercase letters indicate significant differences among different months within each soil depth and different lowercase letters significant differences among different soil depth within each month ($p < 0.05$).

4. Discussion

Fresh seeds of *S. alnifolia* did not germinate in their natural habitat after sowing and none germinated at 5/15 °C, 10/20 °C, 15/25 °C, and 20/30 °C during 2 months of laboratory experiments, indicating that freshly dispersed seeds of *S. alnifolia* are dormant. Seeds of *S. alnifolia* imbibed water quickly, increasing in mass by 70.08% during 12 h of water immersion; in addition, seed coat scarification only led to a slightly greater water absorption rate (speed). We conclude that seeds of *S. alnifolia* have no PY or PY+PD, because PY will result from a water-proof palisade layer(s) within the seed or the fruit coats [4]. In addition, seeds of the *S. alnifolia* possess well-developed embryos under the coverage of a thin layer of endosperm cells, along with a dark brown seed coat, indicating that the embryos of *S. alnifolia* seeds were fully developed and they had not developed MD or MPD. Thus, we can conclude seeds of *S. alnifolia* have PD.

Physiological dormancy has been identified as the vital type of seed dormancy among temperate deciduous forest plants; cold stratification for 26–180 days is necessary for seeds of various species to break dormancy, depending on the species involved [4]. Nikolaeva [20] defined three levels of PD, including non-deep, intermediate, and deep PD. Warm or cold stratification can break non-deep PD, while only cold stratification for relatively long periods can overcome intermediate and deep PD [8]. Applying GA_3 can effectively break non-deep PD, but fails to break deep PD. The use of GA_3 could replace the effects of cold stratification for some species having intermediate dormancy, but not in others. In our study, the overall results show that 150 days of cold stratification is the only effective treatment that overcomes dormancy and enhances germination of *S. alnifolia* seed. Long periods of cold stratification can decrease the contents of endogenous abscisic acid in the seeds, and this will trigger germination [17,21]. This species thus falls more or less in the same group as *S. discolor* in which Nikolaeva [20] reported that a longer period of cold stratification was necessary to overcome deep PD.

Cold stratification of 9–26 weeks has been reported to break dormancy for other *Sorbus* species. Devillez [22] suggested that cold stratification for 6 months was required for obtaining the greatest germination percentage of *S. aucuparia*. Seeds of *S. mougeotii* were deeply dormant, but could germinate at a higher percentage after cold stratification for 32 weeks at 4 °C [23]. Germination might be achieved with cold stratification at 1 °C for *S. glabrescens* [24]. Our result indicates that, following cold stratification for 150 days, seeds of *S. alnifolia* would germinate at 91%, 48%, 13%, and 8%, at 5/15 °C, 10/20 °C, 15/25 °C, and 20/30 °C, respectively. Seed germination will be limited to the period in which germination can overlap field temperatures and the range of temperature [4]. The high germination percentages observed at 5/15 °C and 10/20 °C coincided with the temperatures of April and May in natural habitat. The low germination percentage observed at 15/25 °C and 20/30 °C were possibly caused by secondary dormancy. Similar findings were observed with *Sorbus aucuparia* seeds [25,26] and *S. glabrescens* seeds [24,27].

Warm stratification increased seed germination percentages of *S. alnifolia*. However, seeds of *S. alnifolia* are dispersed during August and September when they could only experience two months of warm stratification before cold stratification occurred. In this study, one month of warm stratification plus cold stratification is superior to cold stratification alone with no previous warm treatment. Because seeds of some *Sorbus* species are also associated with a mechanical dormancy as a result of a hard seed coat [11,28], warm stratification can contribute to breaking down this hard seed coat. Thus, a short warm stratification before cold stratification was proposed to increase the germination percentage of *S. alnifolia* seeds. However, two months of warm stratification combined with cold stratification was not found to be superior to cold stratification alone in terms of breaking the PD of *S. alnifolia*. The possible reason might be that high temperature induced secondary dormancy. Similar results were found in *Acer saccharum* Marshall [29], *Carpinus betulus* L. [30], *C. caroliniana* Walter [31], *Fraxinus angustifolia* Vahl [32], *Prunus dulcis* (Mill.) DA Webb [33], *Sorbus aria* Crantz [22], and *Styrax japonicas* Siebold & Zucc. [34].

It is known that, without cold stratification, embryos that are removed from seeds of various species possessing deep PD will not grow; alternatively, they may grow in an abnormal way [4,28,29]. In *Acer saccharum*, the removed embryo axis could grow when the cotyledons had been excluded [35]. In addition, collected *Crataegus mollis* embryos displayed cotyledon swelling to some degree, but no hypocotyl elongation was detected [35]. For numerous species, especially for some members of the Rosaceae, removing the embryos from the seeds with no cold stratification will lead to abnormal and slow plant growth [4,36–39]. In the present study, no experiments were carried out on excised embryos. However, we found scarifying of the seed coat and partial removal of the cotyledon could significantly increase the germination percentage of *S. alnifolia*. This indicates the seed coat of *S. alnifolia* produces a certain level of mechanical resistance to embryonic growth and especially to radicle growth and elongation. Seed coats were also reported to limit the gas exchange of the embryo, blocking the entry of oxygen and the discharge of carbon dioxide, thus inhibiting respiration [4,33,40,41]. Therefore, such a mechanical obstacle in the seed coat is one of the reasons that leads to the dormancy of *S. alnifolia* seeds. Baskin and Baskin [4] consider the mechanical obstacle of the seed coat is a manifestation of PD, since the breaking of embryonic dormancy through cold stratification would lead to sufficient growth potential in the seed making it able to penetrate the endocarp.

It is known that GA can trigger the sprouting of seeds possessing non-deep as well as intermediate PD; however, GA cannot break the deep PD in the intact transmitted units [4]. Intact *Acer saccharum* samaras had no response to GA or kinetin. Nevertheless, GA and kinetin could lower the sprouting requirements on cold stratification after the samara walls had been resected; moreover, the seed coats were pricked using a pin [29]. In addition, GA could stimulate the development of embryos resected from seeds possessing a deep PD, but in other species, it did not (see Baskin and Baskin [4] for more details on the influence of GA on the sprouting of deep PD).

The soil seed bank in *S. alnifolia* experienced very obvious seasonal changes. In July of the year following seed dispersal, the soil seed bank had disappeared. This indicated that *S. alnifolia* has a transient soil seed bank [42]. Specifically, the soil seed bank only existed in winter; meanwhile, the seeds would germinate in spring. Nonetheless, we found almost no annual seedlings remained under the mother plant during the germination season. This may occur because (1) the seed bank of *S. alnifolia* may be disturbed by animal grazing or pathogens, which decreases the viability of the seeds. Seeds of *S. alnifolia* are much more likely to germinate under high humidity conditions in their natural habitat; the seeds are easily inactivated, which results in an extremely low seed germination percentage. The results of the viability test of *S. alnifolia* seeds also showed that the viability of freshly matured *S. alnifolia* seeds was 87.65 ± 11.67%, but this declined to 38.25% after 6-months of storage at room temperature. (2) Flesh seeds of *Sorbus* species were reported to contain substances that inhibit seed germination [16,43]. Without passing through a bird's digestive tract, few seeds could be germinated in *S. pohuashanensis* [39], and seedlings of *S. aucuparia* that grew from ingested seeds appeared first and grew faster than seeds that had not been ingested [14]. As a result, dormancy leads to this species being rare in the wild.

From an ecological perspective, the long period of chilling required by *S. alnifolia* seeds prevents germination, allowing the seedlings to avoid death in the early stages of growth. *Sorbus alnifolia* is naturally widely distributed, but individuals are widely scattered in relatively high elevation mountains. Strong cold weather resistance and deep seed dormancy allow the species to adapt to cold climates and prevents germination in autumn and winter, while a high-temperature fluctuation following cold stratification can allow the seeds to avoid premature germination in winter. We suggest that such a delay in germination is advantageous by allowing the seedlings to avoid adverse environmental conditions. Thus, the vulnerable seedlings can avoid severe winter conditions by delaying emergence until spring [44].

5. Conclusions

Seeds of *S. alnifolia* have a deep physiological dormancy, and 150 days of cold stratification is the only effective treatment that overcomes dormancy and enhances the germination of *S. alnifolia* seed. The long chilling requirements of *S. alnifolia* seeds would avoid seedling death in winter. Exposure of the seeds to ranges of temperatures at 15/25 °C and 20/30 °C resulted in secondary dormancy. Seed of *S. alnifolia* formed a transient seed bank. The viability of freshly matured *S. alnifolia* seeds was 87.65% ± 11.67%, but this declined to 38.25% after 6-months of storage at room temperature. The information gained from this study should enable horticulturalists and seed ecologists to reduce the time to obtain *S. alnifolia* seedlings and thus provide a useful reference horticultural industry and species conservation.

Author Contributions: K.Z., Y.T. and J.T. conceived and designed the study; K.Z., Y.T. and Y.Z. collected and analyzed the data; Y.T. and J.T. wrote the paper.

Funding: This research was funded by the National Natural Science Foundation of the P. R. China (grant no. 31800340); The Jiangsu Key Laboratory for Horticultural Crop Genetic Improvement of the P. R. China (grant no. 2017023); and the Priority Academic Program Development from the Jiangsu Government.

Acknowledgments: Thanks to the anonymous reviewers for their constructive and valuable comments and the editors for their assistance in refining this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Vázquez-Yanes, C.; Orozco-Segovia, A. Patterns of seed longevity and germination in the tropical rainforest. *Annu. Rev. Ecol. Syst.* **1993**, *24*, 69–87. [CrossRef]
2. Sautu, A.; Baskin, J.M.; Baskin, C.C.; Deago, J.; Condit, R. Classification and ecological relationships of seed dormancy in a seasonal moist tropical forest, Panama, Central America. *Seed Sci. Res.* **2007**, *17*, 127–140. [CrossRef]
3. Herranz, J.M.; Ferrandis, P.; Martínez-Duro, E. Seed germination ecology of the threatened endemic Iberian *Delphinium fissum* subsp. *sordidum* (Ranunculaceae). *Plant Ecol.* **2010**, *211*, 89–106. [CrossRef]
4. Baskin, C.C.; Baskin, J.M. *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*, 2nd ed.; Elsevier/Academic Press: San Diego, CA, USA, 2014.
5. Hu, D.D.; Baskin, J.M.; Baskin, C.C.; Yang, X.J.; Huang, Z.Y. Ecological role of physical dormancy in seeds of *Oxytropis racemosa* in a semiarid sandland with unpredictable rainfall. *J. Plant. Ecol.* **2018**, *11*, 542–552. [CrossRef]
6. Fricke, E.C.; Wright, S.J. The mechanical defence advantage of small seeds. *Ecol. Lett.* **2016**, *19*, 987–991. [CrossRef]
7. De Souza, A.F.; de Andrade, A.C.S.; Ramos, F.N.; Loureiro, M.B. Ecophysiology and morphology of seed germination of the neotropical lowland tree *Genipa americana* (Rubiaceae). *J. Trop. Ecol.* **1999**, *15*, 667–680. [CrossRef]
8. Finch-Savage, W.E.; Leubner-Metzger, G. Seed dormancy and the control of germination. *New Phytol.* **2006**, *171*, 501–523. [CrossRef]
9. Liu, H.J.; Zheng, Y. The beauty of Beijing wildflowers. *For. Hum.* **2018**, *8*, 48–59.
10. Lu, L.D.; Gu, C.Z.; Li, C.L.; Crinan, A.; Bruce, B.; Anthony, R.B.; David, E.B.; Hiroshi, I.; Hideaki, O.; Kenneth, R.R.; et al. Rosaceae. In *Flora of China*; Hong, D.Y., Pan, K.Y., Turland, N.J., Eds.; Science Press and Missouri Botanical Garden Press: Beijing, China; St. Louis, MO, USA, 2003; Volume 9, pp. 46–434.
11. Afroze, F.; O'Reilly, C. Breaking seed dormancy in European rowan seeds and its implications for regeneration. *New For.* **2013**, *44*, 547–557. [CrossRef]
12. Grime, J.P.; Hodgson, J.G.; Hunt, R. *Comparative Plant Ecology: A Functional Approach to Common British Species*; Unwin Hyman: London, UK, 1988.
13. Devillez, F.; Fraipont, J.; Tissot, M. Comportement des embryones isolés de *Sorbus aria* (L.) Crantz, de *S. aucuparia* L. et de *S. torminalis* (L.) Crantz en foction de leurs positions sur le milieu de culture et des prétraitements appliqués aux graines. *Bull. Cl. Sci. Acad. R. Belg.* **1980**, *66*, 162–182.
14. Yagihashi, T.; Hayashida, M.; Miyamoto, T. Effects of bird ingestion on seed germination of *Sorbus commixta*. *Oecologia* **1998**, *114*, 209–212. [CrossRef]
15. Paulsen, T.R.; Högstedt, G. Passage through bird guts increases germination rate and seedling growth in *Sorbus aucuparia*. *Funct. Ecol.* **2002**, *16*, 608–616. [CrossRef]
16. Oster, U.; Bios, I.; Rüdiger, W. Natural Inhibitors of germination and growth IV compounds from fruit and seeds of mountain ash (*Sorbus aucuparia*). *Zeitsch. Naturforsch. Sect. C.* **1987**, *42*, 1179–1184. [CrossRef]
17. Bian, L.; Yang, L.; Wang, J.A.; Shen, H.L. Effects of KNO_3 pretreatment and temperature on seed germination of *Sorbus pohuashanensis*. *J. For. Res.* **2013**, *24*, 309–316. [CrossRef]
18. Wang, J. *Shandong Climate*; China Meteorological Press: Beijing, China, 2005.
19. Zhang, K.L.; Baskin, J.M.; Baskin, C.C.; Yang, X.J.; Huang, Z.Y. Lack of divergence in seed ecology of two *Amphicarpaea* (Fabaceae) species disjunct between eastern Asia and eastern North America. *Am. J. Bot.* **2015**, *102*, 860–869. [CrossRef]
20. Nikolaeva, M.G. *Physiology of Deep Dormancy in Seeds*; NSF: Washington, DC, USA, 1969.
21. Yang, L.; Shen, H.L.; Liang, L.D.; Liu, C.P. Changes in endogenous hormone content in seeds of *Sorbus pohuashanensis* (Hance) Hedl. during artificial drying and cold stratification. *Plant Physiol. Commun.* **2008**, *44*, 682–688.
22. Devillez, F. Influence de la stratification sur les graines et les fruits sur la germination de *Sorbus aria* (L.) Crantz, *S. aucuparia* L. et *S. torminalis* (L.) Crantz. *Bull. Cl. Sci. Acad. R. Belg.* **1979**, *65*, 312–329.
23. Jensen, M. Effect of seed maturity and pretreatment on dormancy and germination of *Sorbus mougeotii* seeds. *Scand. J. For. Res.* **2003**, *18*, 479–486. [CrossRef]

24. Taylor, C.W.; Gerrie, W.A. Effects of temperature on seed germination and seed dormancy in *Sorbus glabrescens* Cardot. *Acta. Horti.* **1987**, *215*, 185–192. [CrossRef]
25. Zentsch, W. Stratification of *Sorbus aucuparia* L. seeds. In *Proceedings of the International Symposium on Seed Physiology of Woody Plants*; Bialobok, S., Suszka, B., Eds.; Panstwowe Wydawnictwo Naukowe: Warsaw, Poland, 1970; pp. 127–132.
26. Lenartowicz, A. Warm-followed-by-cold stratification of mountain-ash (*Sorbus aucuparia* L.) seeds. *Acta. Hort.* **1988**, *226*, 231–238. [CrossRef]
27. Suszka, B. Conditions for the breaking of dormancy and germination of hornbeam (*Carpinus betulus* L.) seeds. In *Proceedings of the International Symposium on Seed Physiology of Woody Plants*; Bialobok, S., Suszka, B., Eds.; Panstwowe Wydawnictwo Naukowe: Warsaw, Poland, 1970; pp. 97–109.
28. Flemion, F. After-ripening, germination, and vitality of seeds of *Sorbus aucuparia* L. *Contrib. Boyce Thompson Inst.* **1931**, *3*, 413–439.
29. Webb, D.P.; Dumbroff, E.B. Factors influencing the stratification process in seeds of *Acer saccharum*. *Can. J. Bot.* **1969**, *47*, 1555–1563. [CrossRef]
30. Takos, I.A.; Efthimiou, G.S. Germination results on dormant seeds of fifteen tree species autumn sown in a northern Greek nursery. *Silvae Genet.* **2003**, *52*, 67–70.
31. Rudolf, P.O.; Phipps, H. *Carpinus* L. Hornbeam. In *Seeds of woody plants in the United States*; Schopmeyer, C.S., Ed.; Agriculture Handbook No. 450; USDA Forest Service: Olympia, WA, USA, 1974; pp. 266–268.
32. Piotto, B.; Piccini, C. Influence of pretreatment and temperature on the germination of *Fraxinus angustifolia* seeds. *Seed Sci. Technol.* **1998**, *26*, 799–812.
33. García-Gusano, M.; Martínez-Gómez, P.; Dicenta, F. Breaking seed dormancy in almond (*Prunus dulcis* (Mill.) DA Webb). *Sci. Hort.* **2004**, *99*, 363–370. [CrossRef]
34. Roh, M.; Bentz, J.A.; Wang, P.; Li, E.; Koshioka, M. Maturity and temperature stratification affect the germination of *Styrax japonicus* seeds. *J. Hortic. Sci. Biotechnol.* **2004**, *79*, 645–651. [CrossRef]
35. Janerette, C.A. An in vitro study of seed dormancy in sugar maple. *For. Sci.* **1978**, *24*, 43–49.
36. Phartyal, S.S.; Godefroid, S.; Koedam, N. Seed development and germination ecophysiology of the invasive tree *Prunus áserotina* (Rosaceae) in a temperate forest in Western Europe. *Plant ecol.* **2009**, *204*, 285–294. [CrossRef]
37. Morgenson, G. Effects of cold stratification, warm-cold stratification, and acid scarification on seed germination of 3 *Crataegus* species. *Tree Planters' Notes.* **2000**, *49*, 72–74.
38. Choi, G.E.; Ghimire, B.; Lee, H.; Jeong, M.J.; Kim, H.J.; Ku, J.J.; Lee, K.M.; Son, S.W.S.W.; Lee, C.H.; Park, J.I.; et al. Scarification and stratification protocols for breaking dormancy of *Rubus* (Rosaceae) species in Korea. *Seed Sci. Technol.* **2016**, *44*, 239–252. [CrossRef]
39. Davis, W.E.; Rose, R.C. The effect of external conditions upon the after-ripening of the seeds of *Crataegus mollis*. *Bot. Gaz.* **1912**, *54*, 49–62.
40. Du Toit, H.J.; Jacobs, G.; Strydom, D.K. Role of the various seed parts in peach seed dormancy and initial seedling growth. *Am. Soc. Hort. Sci.* **1979**, *104*, 490–492.
41. Chen, S.Y.; Chien, C.T.; Chung, J.D.; Yang, Y.S.; Kuo, S.R. Dormancy-break and germination in seeds of *Prunus campanulata* (Rosaceae): role of covering layers and changes in concentration of abscisic acid and gibberellins. *Seed Sci. Res.* **2007**, *17*, 21–32. [CrossRef]
42. Thompson, K.; Grime, J.P. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *J. Ecol.* **1979**, *67*, 893–921. [CrossRef]
43. Shen, H.L.; Yang, L.; Zhang, J.Y.; Feng, D.D.; Fan, S.H. Influencing factors to seed dormancy and germination characteristics of *Sorbus pohuashanensis*. *Sci. Silva. Sin.* **2006**, *42*, 133–139.
44. Vandelook, F.; Bolle, N.; van Assche, J.A. Multiple environmental signals required for embryo growth and germination of seeds of *Selinum carvifolia* (L.) L. and *Angelica sylvestris* L.(Apiaceae). *Seed Sci. Res.* **2007**, *17*, 283–291. [CrossRef]