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# Influence of Temperature, Moisture, and Light Conditions on the Germination of Seeds of *Erythronium japonicum* Decne

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# **Abstract**

*Erythronium japonicum* is a representative species of spring ephemeral, forest floor plants in substitution forests in Japan. Seed germination tests were conducted to observe the effects of temperature, light, and moisture content on the germination of E. japonicum. E. japonicum seeds did not germinate at constant temperatures of 5°C, 10°C, 15°C, 20°C, or 25°C. Seeds germinated at 5°C and 10°C after storage at 20°C for 1 or 2 months. Seed germinability was affected by desiccation to only 40% moisture content. Seeds germinated well in both light and dark conditions. Conditions under the leaf litter layer showed unique characteristics of temperature, light, and moisture content. The temperature was around 20°C under the leaf litter layer, even though it was 30°C above. The moisture content under the leaf litter layer was higher than that above. The leaf litter blocked out the sunlight. The temperature of 20°C necessary for E. japonicum germination occurred below, but not above, the leaf litter layer. The avoidance of decreasing in germinability from reduced seed moisture content in a short time is consistent with the high moisture conditions under the leaf litter layer. The leaf litter layer plays an important role in providing optimal conditions of temperature, light, and moisture content for the germination of *E. japonicum* seeds.

#### **Keywords**

*Erythronium japonicum*, Leaf Litter Layer, Life History, Seed Germination, Spring Ephemeral

#### 1. Introduction

Satoyama woods are located in the suburbs between mountainous and urban

areas in Japan. Until the 1950s, people traditionally used many plants from Satoyama woods (Morimoto, 2011; Yokohari & Bolthouse, 2011). For example, Quercus acutissima and Q. serrata were used as charcoal fuel, and mother wood to cultivate shiitake (mushrooms). Q. dentata was used for covering food, or caching. Abies firma was used for furniture. Erythronium japonicum and Allium victorialis var. platyphyllum were used for food. Their leaves were used for organic fertilizer or fuel. Satoyama woods have not been used as natural resources since people began using oil fuels as energy sources and their general lifestyles changed. The area of open space with plants has decreased at a rapid pace owing to unrecognized economical aspects (Iida & Nakashizuka, 1995; Stéphanie et al., 2007), and the abandoned Satoyama woods are no longer balanced between succession and felling for human uses. Substitution forests consist of deciduous trees that have been replaced by evergreen broadleaved forest (Nakajima et al., 2017). Substitution forest in Satoyama woods has decreased the populations of forest floor plants (Nakajima et al., 2016).

Forest floor plants that were used as foods or habitats in previous years are mainly used for tourism now. However, open spaces with many plants and animals have additional functions related to recreation, air pollution, disaster prevention, evacuation, habitats, and biodiversity. Forest floor plants are not only useful for the conservation of native plants, but also provide beautiful landscapes in urban areas or suburbs.

E. japonicum has a unique arrangement of leaves and flowers and is a spring ephemeral, forest floor plant (Kawano, 2004). E. japonicum had been used as a food source, and is very popular among the local people. However, the area of distribution of E. japonicum has decreased due to development of housing and other buildings, or abandonment of the subsistence forest. Numbers of E. japonicum have decreased year on year, and it is now designated as Vulnerable (VU), which includes species at increased risk of extinction in 16 prefectures in Japan at RDB (Association of Wildlife Research and EnVision, 2007).

This study was conducted to identify favorable conditions for the propagation of *E. japonicum* by examining seed germination tests in relation with temperature, light, and moisture content.

# 2. Materials and Methods

#### 2.1. Seeds Selection

Seeds used for germination tests at different temperatures and light conditions were collected on June 4, 2001 [Fukushima 2001], and May 31, 2002 [Fukushima 2002] at Koriyama city, Fukushima Prefecture (37°24'46.31", 140°16'22.83"), and June 20, 2005 [Akita 2005], and June 3, 2007 [Akita 2007] at Nishiki town, Senboku city, Akita Prefecture (39°41'16.57", 140°33'1.80"), respectively. Seeds used for germination tests of moisture content were [Akita 2007] and collected on June 7, 2008 [Akita 2008] at Nishiki town, Senboku city, Akita Prefecture, and June 5, 2008 [Fukushima 2008] at Koriyama city, Fukushima Prefecture.

The main vegetation where *E. japonicum* grows at Koriyama city, Fukushima Prefecture was *Quercus serrata*, *Q. acutissima*, *Styrax japonica*, *Ilex macropoda*, *Pinus densiflora*, *Swida controversa*, *Acer amoenum* var. *matsumurae*, and *Zelkova serrata*. The area of Nishiki town, Senboku city, Akita Prefecture was the main field for *Castanea crenata* fruit collection. The soil types of both Fukushima Prefecture and Akita Prefecture were same as brown forest soil.

## 2.2. Germination Tests in the Laboratory

Thirty seeds were placed in 9-cm diameter glass dishes with two sheets of filter paper soaked in 4 ml tap water. Dishes were placed in individual chambers (NK System Co.) maintained at 10°C, 15°C, 20°C, or 25°C and illuminated using a fluorescent lamp on a 12-h light cycle (28.7 µmol/m²/S). Seed germination tests were conducted with three replicates. Germinated seeds were recorded and removed from the dish each week and the percentage of germinated seeds was calculated until germination ceased (1-year maximum). Surface sterilization for the seed germination tests did not do.

Temperature, light, and moisture content differs between above and below the leaf litter layer (Suzuki, 2006). In this study, to observe seed germination at different temperatures, seed germination tests were conducted at  $10^{\circ}$ C,  $15^{\circ}$ C,  $20^{\circ}$ C, and  $25^{\circ}$ C immediately after seed collection and after storage at room temperature ( $20^{\circ}$ C -  $25^{\circ}$ C) for 1 to 3 months at first. These seeds were stored in wetted perlite after being put in a net bag ( $10 \text{ cm} \times 10 \text{ cm}$ ) in an unglazed pot (23 cm diameter, 20 cm depth). Seed germination tests were conducted under light conditions in same above individual chambers (NK System Co.) with seeds [Fukushima 2001]. One of the seeds was stored in dry conditions to observe the influence of desiccation on seed germination, as below.

Secondly, seeds [Fukushima 2002] were used in germination tests at 10°C, 15°C, 20°C, and 25°C immediately after seed collection, after storage at 5°C or 20°C for 3 months, and after storage at 20°C for 3 months followed by 5°C for 3 months under light conditions. The seeds were stored in wet conditions, packed in polyethylene bags for 1 to 3 months. One of the seeds was stored in dry conditions until desiccated to 16% M.C. (moisture content) to observe the influence of desiccation on germination, as below.

Thirdly, seed germination tests were conducted at a monitoring temperature representing the soil above the leaf litter layer (30°C or higher) for 2 months immediately after seed collection on [Akita 2005]. The monitoring temperature followed Suzuki & Kawano (2010), who showed that temperature above the leaf litter layer occasionally reached over 30°C; however, temperature below the leaf litter layer was more constant at approximately 22.5°C.

During one seed germination tests, we looked the cross sections of seeds to observe embryo development after seed collection, and calculate the ratio of embryo to endosperm. The seeds used were [Akita 2007], and stored at 20°C for 1 month, followed by 10°C for 1 month, and 5°C under light condition. To observe the cross sections of seeds, we cut 10 samples at intervals of 2 weeks and took pictures using a

stereoscopic microscope (Nikon SMZ800, Tokyo, Japan). Ratio of area of embryo to endosperm was calculated by measuring area with planimeter.

Fourthly, germination tests under different light conditions were conducted using seeds [Akita 2007]. Glass dishes were used for light conditions, and stainless-steel dishes were used for dark conditions, respectively. The germination tests were done at 5°C after 20°C for 1 month and 10°C for 1 month, or 10°C after 20°C for 1 month only. When we moved seeds to 5°C or 10°C from storage conditions, seeds that had changed from brown to black, or seeds that were empty were discarded. After seed germination, we continued to observe the growth of cotyledons at monitoring temperatures for natural conditions as leaf litter layer at forest floor from 7.5°C to 2.5°C.

Fifthly, observe the effect of desiccation on germination, germination rates of seeds dried at room temperature were compared with non-desiccated seeds that were [Fukushima 2001] and [Fukushima 2002]. Seeds [Fukushima 2001] were tested at 10°C, 15°C, 20°C, and 25°C after seed desiccation and compared with seeds stored in wet conditions in a polyethylene bag. Seeds [Fukushima 2002] were also used for germination tests at 5°C, 10°C, 15°C, and 20°C after storage at 20°C for 3 months and desiccated to M.C. 16%, to compare with seeds stored in wet conditions.

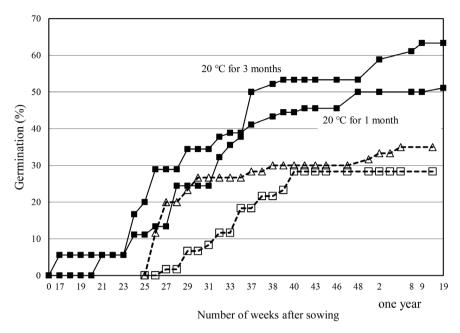
Finally, the relationship between seed M.C. and germination was investigated using seeds [Akita 2007] and [Akita 2008], and [Fukushima 2008]. These germination tests were conducted at 5°C after desiccation at 20°C for 2 months.

#### 3. Results

## 3.1. Germination in Different Temperatures and Light Conditions

Seeds [Fukushima 2001] and [Fukushima 2002] showed lower germination rates immediately after seed collection (**Figure 1** and **Figure 2(a)**). No seeds germinated at 20°C or 30°C. The seeds from [Fukushima 2001] stored at 20°C for 1 or 3 months germinated at a rate of 50% to 60%, respectively (**Figure 1**).

The seeds [Fukushima 2002] stored at 5°C for 3 months showed a germination rate of 37% only at 10°C, which was half the germination rate of seeds stored at 20°C for 3 months (Figure 2(b)). These seeds did not germinate at 15°C or 20°C after storage at 5°C, or at 25°C after storage at 20°C for 3 months (Figure 2(b)). Seeds stored at 20°C for 3 months (Figure 2(b)) and seeds stored at 5°C for 3 months after storage at 20°C for 3 months (Figure 2(c)) showed a high germination rate of 60% at 10°C. The germination rates of seeds kept at 10°C and 15°C after storage at 20°C for 3 months were not different from seeds stored at 5°C for 3 months, after storage at 20°C for 3 months (Figure 2(b)) and Figure 2(c)). As shown in Figure 7, although the germination rates were slightly different, the final percentage of germination was the same between seeds stored at 20°C for 1 months, then 10°C for 1 month, then moved to 5°C, and seeds stored at 20°C for 1 month then moved to 10°C. Thus, *E. japonicum* seeds germinate well at 5°C or 10°C after experiencing a warm temperature of 20°C for 3 months.



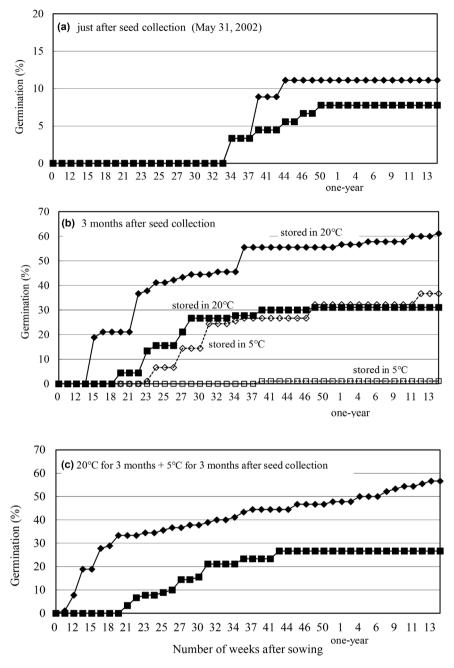
**Figure 1.** Germination curves for *E. japonicum* seeds [Fukushima 2001]. Seeds were stored in wetted perlite for "wet conditions" or a paper bag for "dry conditions" at room temperature (20°C - 25°C). Seed germination tests were conducted under light conditions at triangle Δ: 10°C and squares ■ & □: 15°C. The germination tests were done on just after seed collection (dotted line and white symbols), and begun on July 11, or Sep. 21 after storage in 20°C (line and black symbols). There was no experiment at 10°C after storage in 20°C under wet condition. Seed germination rate represents the average of three replicates. Seeds that did not germinate immediately after seed collection at 20°C or 25°C. Seeds were stored for 1 month until July 11, 2001, and for 3 months until September 21, 2001, did not germinate at 20°C or 25°C. Seeds stored in dry conditions at room temperature showed 3 germination percentages at 10°C.

However, seeds stored at 30°C showed half the germination rate of seeds stored at 20°C for 2 months after seed collection. Or seeds tested at 5°C immediately after collection showed little germination (**Figure 3**). The seeds tested at a monitoring temperature for the above leaf litter layer showed 8% germination rate, compared with seeds tested at a monitoring temperature for under the leaf litter layer, 33% (**Figure 4**).

In these temperate experiments, the embryos grew at 20°C for 1 month, 10°C for 1 month, and then at 5°C (**Figure 5** and **Figure 6**). As shown **Figure 7**, there was no difference in seed germination rate between light and dark conditions. The cotyledons grew until early May under monitoring leaf litter layer temperature from winter to spring after seed germination (**Figure 8**).

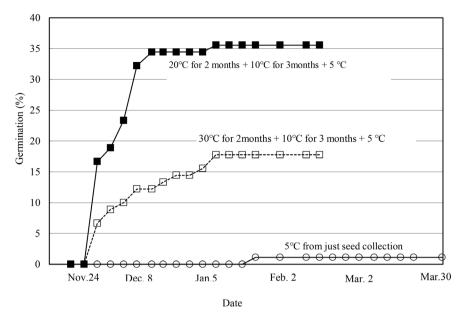
#### 3.2. Germination in Dry Conditions

Desiccated seeds [Fukushima 2001] showed 3% germination at 10°C after storage at room temperature, 20°C - 25°C (**Figure 1**). Also, seeds desiccated to 16% M.C. did not germinate at temperatures of 10°C, 15°C, 20°C, or 25°C after storage at 20 °C for 3 months, as shown in **Figure 2**. The relationship between M.C.

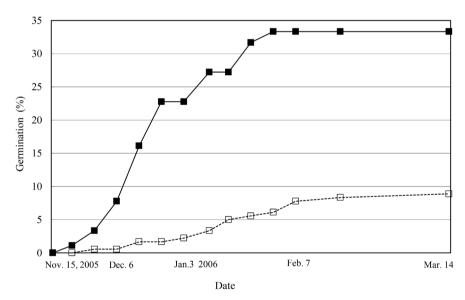


**Figure 2.** Germination curves for *E. japonicum* seeds. Seeds [Fukushima 2002] stored in wet conditions in a polyethylene bag. Seed germination tests were conducted under light conditions at ◆, ♦: 10°C and ■, □: 15°C. Not all seeds germinated at 20°C or 25°C. The seed germination rates represent the average of three replicates. A: immediately after seed collection. B: seeds were stored at 5°C (♦, □, and dotted line); at 20°C (•, ■, and line) for 3 months. The seeds did not germinate at 15°C and 20°C after storage at 5°C, and at 25°C after storage at 20°C for 3 months. Seeds dried to 16% moisture content did not germinate at 10°C, 15°C, 20°C or 25°C after storage at 20°C. C: seeds stored at 5°C until testing (November 25, 2002) in addition to storage at 20°C until August 12, 2002.

and seed germination rate for the three kinds of seeds source showed that seeds decreased and lost germinability at less than 60% and 40% M.C., respectively (Figure 9).



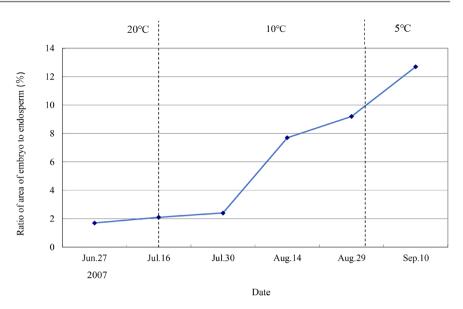
**Figure 3.** Germination curves for *E. japonicum* seeds [Akita 2005]. Germination tests were conducted ○: at 5°C immediately after collection; •: at 5°C after storage at 20°C from seed collection (June 30) to August 29, followed by 10°C from August 29 to November 26, 2005 and □: at 5°C after storage at 30°C from seed collection (June 30) to August 29, and 10°C from August 29 to November 26, 2005.



**Figure 4.** Germination curves at different temperatures for *E. japonicum* seeds [Akita 2005]. ■: monitoring temperature as below the leaf litter layer; □: monitoring temperature as above the leaf litter layer.

### 4. Discussion

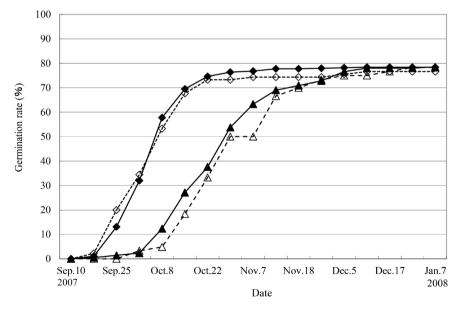
E. japonicum seeds need a temperature of about 20°C during the 3 months before germination at 5°C to 10°C. Seed germination rates were low at the monitoring above leaf litter layer temperature and high temperature (30°C) experience for 2 months immediately after seed collection. Seeds that were



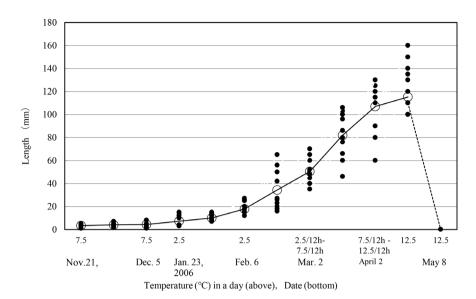
**Figure 5.** The ratio of embryo to endosperm (%) during storage at 20°C for 1 month, 10°C for 1 month, and at 5°C in seeds [Akita 2007].



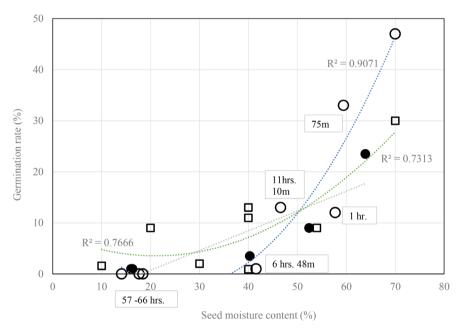
**Figure 6.** Embryo (arrows) in seeds stored in 20°C for 1 month (left), and after 20°C for 1 month and 10°C for 1 month (right) of seeds [Akita 2007].



**Figure 7.** Germination curves at different temperatures and light conditions for *E. japonicum* seeds [Akita 2007]. Line is light and dotted line is dark conditions. Seeds were stored at 20°C for 1 month, 10°C for 1 month, and then moved to 5°C ( $\bullet$  and  $\diamondsuit$ ); 20°C for 1 month, and then moved to 10°C ( $\blacktriangle$  and  $\vartriangle$ ).



**Figure 8.** Radicle length after germination in *E. japonicum* seeds [Akita 2005]. The radicle disappeared on May 8, 2006. ○: average; •: individual data points.



**Figure 9.** Relationships between moisture content and germination rate. Seeds for experiments were [Akita 2007] (○), at [Akita 2008] (□), and [Fukushima 2008] (●). Number is hour(s) and minutes after seed desiccation for seeds [Akita 2007] (○).

maintained at 5°C after seed collection also did not germinate. Embryos developed at 10°C after storage at 20°C for 1 month. These results showed that *E. japonicum* seeds germinated well at 5°C - 10°C after experiencing 20°C. Even though M.C. was 40%, the seeds rapidly lost germinability. Under light and dark conditions, there was no difference in seed germination.

The surface below the leaf litter layer, where many seeds are dispersed by myrmecochory, has specific temperatures, light (dark), and moisture conditions:

1) temperature under the leaf litter layer was below 25°C, even in the summer when the temperature above was about 35°C; 2) temperature under the leaf litter layer in the winter remained at 2°C - 4°C, even when the temperature above was below 0°C (Suzuki & Kawano, 2010); 3) high moisture content was maintained under the leaf litter layer even when it was dry above the leaf litter layer (Suzuki, 2006; Young & Evans, 1989); 4) it was dark under the leaf litter layer, since the sunlight was obstructed by the leaf litter (Suzuki, 2006), or plots with leaf litter had significantly lower light levels at the soil surface than those with no litter (Reynolds et al., 2001).

Almost fruits or seeds are fructified in autumn in Japan. *E. japonicum* is a representative species of spring ephemerals in forest floor plants. Fruits or seeds of *E. japonicum* are fructified in late spring or early summer, from late May to early June. *E. japonicum* seeds are heavy with high moisture content on the just after fructification (2.7 g/100 seeds, M.C. 65.3%, Suzuki, 2006). Myrmecochory is when seeds with an elaiosome are dispersed by ants or ground beetles (Culver & Beattue, 1978; Ohara & Higashi, 1987). Many seeds became submerged under the leaf litter over time. We observed seeds that fell under the leaf litter layer at the time when ants or ground beetles eat elaiosomes, or when the leaf litter was disturbed by rain or wind. When we sieved the seeds and leaf litter, even broadly through fingers, these seeds fell under the leaf litter. We also observed the radicle of *E. japonicum* under the leaf litter, and the cotyledon growing through the litter when it emerged (Suzuki, 2013). These results showed that *E. japonicum* seeds were dispersed under the leaf litter layer.

The temperature (20°C) needed to promote germination of *E. japonicum* seeds matched the temperature under the leaf litter layer, but not the temperature above; thus, germinability was reduced. Such a decrease in germinability from such a small loss in seed moisture content and over such a short time is coincident with the moisture conditions above the leaf litter layer. The leaf litter layer maintains the seed moisture content and germinability. Although there is no light under the leaf litter layer, *E. japonicum* seeds can germinate in the dark. Consequently, the seedlings in early spring, late May to early April, under natural conditions are those that germinated under and broke through the leaf litter layer.

The relationships between temperature, light, and moisture conditions in the leaf litter layer and germination of *E. japonicum* follow those for *Trillium apetalon* Makino (Suzuki & Kawano, 2010). These may be the general germination characteristics of spring ephemerals.

Many species of forest floor plants, *Heterotropa muramatsui* (Makino) F. Maek. (*Asarum muramatsui* Makino), *E. japonicum*, and *Cephalanthera falcata* (Thunb.) Blume etc. have recently been listed in the Red Data Book. Spring ephemerals have a unique life history in the specific temperature, light, and moisture conditions of the leaf litter. As a representative spring ephemeral species, *E. japonicum*, takes more than 8 years to produce flowers (Kawano et al., 1982; Kawano, 2004). Therefore, it is more difficult to maintain habitat for *E.* 

*japonicum* over a long time in warm-temperate zones. Previously, people managed the forest floor, e.g. cutting shrubs and *Pleioblastus chino* Makino (Nakajima et al., 2017), and gathering leaves. Moreover, these plants show unique seed germination habits. Warm temperatures which do not reach 30°C in summer, and high seed moisture content facilitate germinability. Leaf litter is very important for the germination of *E. japonicum* seeds. There are some discussions about the role of leaf litter in seed germination (Baskin & Baskin, 2014). When we collected leaf litter in one of the areas for one winter to make compost, there was no influence on germination, since areas of coppiced forest were substantial in the past. However, careful maintenance is needed to maintain forest floor plants in the small areas of fragmented and isolated remaining forests now. To artificially propagate *E. japonicum*, we need to confirm the role of leaf litter in germination.

For the sustainable development of forests, or to benefit from sustainable forest management, we need to maintain enough natural open spaces in suburban areas. It is beneficial to grow forest floor plants to create landscapes, evaluate their recreational functions, and admire natural landscapes (open spaces).

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