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Seed germination of *Pinus koraiensis* Siebold & Zucc. in response to light regimes caused by shading and seed positions

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Abstract

Pinus koraiensis Siebold & Zucc. (Korean pine), the dominant tree species in the mixed broadleaved Korean pine forests (regional climax), is severely restricted by its regeneration failure. To determine the effects of light regimes on *P. koraiensis* regeneration, the seed germination process was examined in shade houses and forest stands (before and after leaf expansion) with various light levels created by shading and seed positions. Despite the large size of *P. koraiensis* seeds (500-600 mg), both light intensity and quality significantly affected the germination percentage in both shade houses and forests. Substantial changes in light intensity and quality led the majority of seeds (80%) to germinate in leafless forests and shade houses, while only a minority (≤ 20%) germinated after leaf expansion in the forests. Moreover, seed germination in shade houses and leafless forests exhibited similar patterns; they consistently reached a 70% shading degree, which was optimal for the seed germination of *P. koraiensis* on topsoil. Seed positioning significantly affected germination for each shading degree, especially when litter and soil coverings drastically inhibited germination. In conclusion, (1) when seeds were not stressed by temperature and moisture, light irradiance played a critical role in the seed germination of *P. koraiensis*; (2) seed positioning, in relation to alterations in light intensity and quality, affected the germination of *P. koraiensis*; (3) a shade house experiment using neutral cloth provided an applicable and controllable way to monitor the *P. koraiensis* seed germination in early spring before leaf expansion. The light requirement for the germination of *P. koraiensis* played a key role in the regeneration of *P. koraiensis* throughout the temperate secondary forests.

Key words: light intensity; mixed broad-leaved Korean pine forests; shade house; seedling recruitment; seed regeneration; leaf expansion.

Resumen

Germinación de semillas de *Pinus koraiensis* Siebold & Zucc en respuesta a regímenes de luz causados por sombreo y situación de la semilla

Pinus koraiensis Siebold & Zucc. es la especie arbórea dominante en los bosques mixtos de frondosas de pinos coreanos (clímax regional) que se ve seriamente limitada por su falta de regeneración. Para determinar los efectos de los regímenes de luz sobre la regeneración de *P. koraiensis* se ha examinado el proceso de germinación de la semilla en cámaras de sombra y en masas forestales (antes y después de la expansión de las hojas) con diferentes niveles de luz creados por sombreado y por la localización de las semillas. A pesar del gran tamaño de las semillas de *P. koraiensis* (500-600 mg), tanto la intensidad de luz y la calidad han afectado significativamente el porcentaje de germinación tanto en cámaras de sombra como en los bosques. Cambios importantes en la intensidad de la luz y en su calidad ocasionaron la germinación de la mayoría de las semillas (80%) en los bosques sin hojas y cámaras de sombra, mientras que sólo una minoría (≤ 20%) germinaron después de la expansión de las hojas en el bosque. Por otra parte, la germinación de las semillas en las cámaras de sombra y en bosques sin hojas mostraron patrones similares. Constantemente han alcanzado un grado de sombreado del 70%, lo cual es óptimo para la germinación de las semillas de *P. koraiensis* en tierra vegetal. La posición de la semilla afecta significativamente a la germinación en cada grado de sombreo, especialmente cuando los revestimientos de desechos vegetales y de tierra inhibieron drásticamente la germinación.

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Como conclusiones, (1) cuando las semillas no se estresan por temperatura o humedad, la irradiación de luz juega un papel crítico en la germinación de las semillas de *P. koraiensis*; (2) La posicion de las semillas, en relación con alteraciones en la intensidad de la luz y la calidad, afectó la germinación de *P. koraiensis*, (3) un experimento en cámara de sombreo utilizando un paño neutral proporciona una manera para controlar la germinación de *P. koraiensis* en primavera, antes de la expansión foliar. El requisito de luz para la germinación de *P. koraiensis* juega un papel clave en la regeneración de los bosques secundarios templadas.

Palabras clave: intensidad de luz; bosques mixtos de pino coreano; cámara de sombreo; reclutamiento de semillas.

Introduction

As one of the most important five-leaved pine species in the northern hemisphere, Korean pine (*Pinus koraiensis* Siebold & Zucc.) is mainly distributed throughout Northeast Asia's (Ma and Zhuang, 1992) mixed broadleaved Korean pine forests (MBKPF). MBKPF, a regional climax vegetation type in the mountainous areas of Northeast China, is composed of *P. koraiensis* and broadleaved tree species, such as *Juglans mandshurica* Maxim., *Fraxinus mandshurica* Rupr., *Tilia* spp. and *Quercus mongolica* Fisch.. Due to an intense amount of human disturbance, as well as abnormally extreme, natural disasters, most of the MBKPF (accounting for 52.5% of Northeast China) have been destroyed and replaced by secondary forests (Wu *et al.*, 2004). Although it has been a goal for the State Forestry Administration to recover the biodiversity of MBKPF in Northeast China, the growth of *P. koraiensis* in the natural forests has been severely restricted by the failure of its natural regeneration (Li *et al.*, 1989).

There are four critical stages necessary for the successful natural regeneration of a species: seed resourcing, successful seed germination, seedling emergence, and survival (Zhu *et al.*, 2003). Based on our preliminary investigation (Yu *et al.*, 2006), regenerated *P. koraiensis* seedlings emerged from deciduous secondary forests with heterogeneous distribution patterns. This phenomenon may largely be due to the responses of seed germination and/or seedling survival of environmental variations induced by the shading degree and seed positioning in the soil. A similar phenomenon was observed for *Larix olgensis* Henry —rather than the germination percentage of *L. olgensis*— as seed survival rates varied with canopy openness and site preparation (Zhu *et al.*, 2008). However, as *P. koraiensis* seedlings are capable of surviving under dense canopies (Hao *et al.,* 2007), the regeneration pattern of *P. koraiensis* seedlings may be primarily due to seed germination variations under different environmental conditions.

Seed germination is the most sensitive stage in the natural regeneration process (e.g., light intensity, light composition, temperature and moisture conditions) (Kyereh *et al.*, 1999; Du *et al.*, 2007) and is sensitive to environmental conditions. In frequently disturbed environments, the microclimates for seed germination change spatially and temporally because of various light transmittances through gaps or canopy openness. The formation of gaps noticeably contributes to a significant increase in irradiance at ground level (Zhu *et al.*, 2003). Furthermore, temperature, moisture and other site conditions always vary with alterations of incident light quantity and intensity, which in turn, depend on canopy openness (Rincón and Huante, 1993; Pearson *et al.*, 2002). In addition, it was found that light and subsequent temperature signals in early spring prior to leaf expansion were also higher or nearly identical to those in the gaps (Seiwa *et al.*, 2009). In natural habitats, and from leafless period to leaf period, the shading elements shifted from tree trunks and branches to the dynamics of leaves within the canopy, which induced variations of light intensity (Photosynthetic Photon Flux Density, PPFD) and quality (Red/Far red light ratio, R/FR) (Griffith *et al.*, 2005; Seiwa *et al.*, 2009). In leafless forests (before leaf expansion), light quality does not change a great deal; thus, the light intensity may be more important in influencing seed germination. With the decrease of canopy openness (after leaf expansion), the composition of light spectra varies greatly after transmittance through the canopy leaves (Holmes and Smith, 1977; Pons, 1983), as tree leaves prefer a specific light spectrum (red/blue light).

Generally, various seed positions can occur during seed maturation, including seed positioning on top of litter/soil, into the soil, and/or beneath the cover of litter and soil. Therefore, besides the shading of canopy, litter and/or topsoil is considered as second filter of light irradiance, which alters germination conditions for seeds beneath them. Further fine-scale heterogeneity in germination sites is induced by the interaction between seed positioning and the variation of canopy openness (Molofsky and Augspurger, 1992). As demonstrated in previous research, litter has had both positive and negative effects on seed germination (Williams *et al.*, 1990; Smith and Capelle, 1992; Xiong and Nilsson, 1999; Muscolo and Sidari, 2006). Litter coverage can lead to alterations of radiation and moisture, which then influences the germination of seeds (Facelli and Pickett, 1991).

Currently, much attention is being paid to the seed germination responses of *Pinus* spp. to light and water conditions. For example, Parker *et al.* (2006) concluded that both germination and emergence were significantly higher under 13% of full sunlight than under 47% of full sunlight for *Pinus strobus* L., another five-leaved pine in the northern hemisphere. Light plays a more critical role than water conditions during seed germination and seedling survival of *Pinus pinaster* Ait. (Ruano *et al.*, 2009). Through controlling the sowing depth, Castro *et al.* (2002) reported that the physical barrier created by an herbaceous layer was the main block for *Pinus sylvestris* L. regeneration, as well as to the potential expansion of the forest. These three species, *P. strobus*, *P. pinaster* and *P. sylvestris*, are known as light-demanding species. However, little information is available on the influence of light conditions (especially the integrated effects of shading and seed position) on the germination of *P. koraiensis*. In the present study, seed germination of *P. koraiensis* was examined in shade houses and forest stands with various shading levels and sowing position treatments.

The primary objectives of this study were to: (1) find out the effects of variations in light characteristics caused by different canopy openness on seed germination of *P. koraiensis*; (2) define the effects of different seed positioning on the seed germination of *P. koraiensis*; (3) determine whether the results observed in forests were in accord with those in shade houses. Finally, some suggestions for improving the regeneration of *P. koraiensis* through regulations of environmental conditions are put forward.

Materials and Methods

Study site

The study was conducted at Qingyuan Experimental Station of Forest Ecology (QESFE) of the Chinese Academy of Sciences, in Liaoning Province, Northeast China (41°51.102' N, 124°54.543' E, 456-1,116 m a.s.l.). The climate, a continental monsoon type, is defined by windy springs, warm and humid summers, and dry and cold winters. The annual average precipitation in the area is 810.9 mm, with a mean annual air temperature of 4.7°C. The maximum temperature is 36.5°C in July, while the minimum is –37.6°C in January. The frost-free period lasts one hundred and thirty days, and the growing season ranges from early April to late September (Zhu *et al.*, 2006). The vegetation is representative of the naturally regenerated secondary forest in this region, dominated by *Pinus koraiensis* Siebold & Zucc., *Larix olgensis* Henry, *Juglans mandshurica* Maxim., *Phellodendron amurense* Rupr., *Fraxinus mandshurica* Rupr., *Quercus mongolica* Fisch., and *Fraxinus rhynchophylla* Hance.

Seed collection and pretreatment

In late September 2009, fresh seeds of *P. koraiensis* were collected from at least five recently fruited trees growing in secondary forest ecosystems at QESFE. From November 2009 to April 2010, approximately 8,000 seeds were kept in the dark and dry storage. Remnant seeds with the same number were mixed with wet sand and buried under ground at low temperatures (–5-0°C) to simulate the transition from cold winter to warm spring. In May 2010, the buried seeds were sterilized by soaking for thirty minutes in a 0.5% potassium permanganate (K_2MnO_4) solution. The floating seeds were subsequently removed. The seeds that sank immediately were, however, considered to be viable (Tanaka-Oda *et al.*, 2009). These viable seeds were of similar size (500-600 mg) to those used for the germination experiment in this study.

Experiment design

Shade house experiments

Aiming to provide light spots, shading was achieved by suspending different layers of black nylon netting above and around each plot. Furthermore, a distance of three centimeters was left between the nylon edge and the ground to permit constant air movement in the shade houses. Prior to the determination of the shading degree design, we placed nine representative sample plots into the secondary mixed forests and monitored the canopy openness variation from April to September by taking monthly fish-eye photos (Fig. 1). Given that all of the potential light regimes appeared frequently in natural forests throughout the entire growing season (Fig. 1), five levels of shading treatments were established, i.e., 0%, 40%, 70%, 85% and 95% shading degree (shading degree = 1 – relative light irradiance). The relative light irradiance in each treatment was calculated with respect to full sunlight values.

Under each shading degree, *P. koraiensis* seeds were sown in five possible seed positions (i.e., LT: on top of the litter; ST: pressed half-way into the soil; LS: beneath the litter of 4 cm depth; S1: under 1 cm soil without litter cover; and LS1: under 1 cm soil, with 4 cm litter coverage) observed in natural forests. Each treatment had forty seeds and was replicated three times. Forest soil was sieved through ca. 3 mm to exclude the residual root or seeds and was dried in the air prior to use. The litter was collected from the secondary mixed forest and dried at ambient temperature for a week before the experiment. The light intensity and quality beneath the litter/soil were measured by placing the light sensor under a slice of transparent glass with litter/soil on top of it. The experiment began in May 2010, and the number of germinated seeds was recorded at three or five-day intervals until the experiment was terminated in October 2010 when no further germination occurred for at least two weeks. Germination was defined as the first needle sprout becoming visible (Xiong and Nilsson, 1999; Argyris *et al.*, 2008; Zhu *et al.*, 2008).

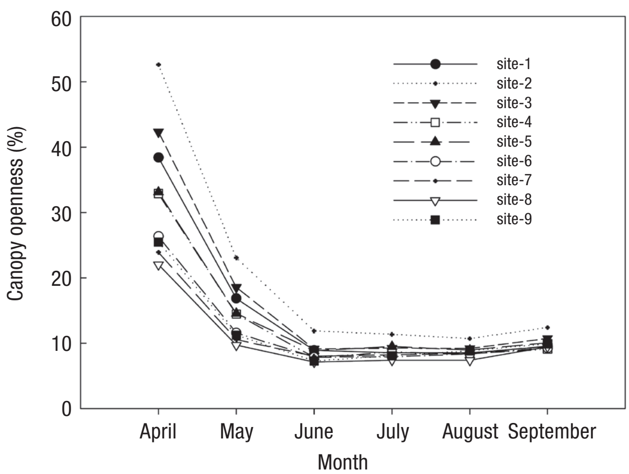

Figure 1. Seasonal variation of canopy openness in broad-leaved mixed forests observed from 9 representative sites.

With a quantum light sensor and a temperature probe connected to a Watchdog Data Logger (Watchdog Model 200, Spectrum Technologies, Inc., Plainfield, IL, USA), the light intensity and the topsoil (0-5 cm) temperature were measured at fifteen-minute intervals on least one sunny day (from 07:00 to 18:00) each month at each replicate site during the germination period (Table 1). Simultaneously, relative humidity measurements were collected with a HOBO Pro (Model H08-032-08, Onset Computer Corp., Bourne, MA) in each shade house. Due to the air cycling design, the relative humidity in each shading treatment was similar and identical to our survey data (Table 1). To compare the temperature variations for different seed positions, the temperature of each treatment was measured repeatedly with a meteorological thermometer in July, at two-hour intervals from 07:00 to 18:00. Light quality was quantified by averaging the measurements from 07:00 to 18:00 on one sunny day of each month with those from another quantum sensor (Skye 110 Red/Far-Red light sensors, Skye instruments Ltd. Llandrindod Wells, Powys, UK) taken at two-hour intervals (Table 1).

Forest experiments

To further investigate seed germination in natural deciduous mixed forests throughout the entire growing season, we also sowed the dry storage seeds in late autumn (before leaf expansion), and the buried seeds in late spring (after leaf expansion) 2010, under seven different canopy openness conditions (before leaf expansion: 0%, 55%, 69%, 76% shading degree; the same sites after leaf expansion: 85%, 90%, 95% shading degree). Thus, we can investigate the germination before and after leaf expansion based on dry storage seeds and buried seeds, respectively. Additionally, five seed positions (LT, ST, LS, S1, and LS1) were involved in each canopy openness condition. Three replicates were used for each shading degree and seed position treatment. The survey lasted two consecutive years. The germination percentage of the seeds was also monitored at five-day intervals. The final germination percentage was calculated when no further germination was recorded for at least two weeks. The microclimate (e.g., light intensity, temperature) for each treatment was only measured after leaf expansion. Light and temperature were monitored after leaf expansion synchronously with those observed in shade houses.

Table 1. Mean light intensity (PPFD), light quality (R/FR), temperature and relative humidity for different treatments in shade houses (A), and in forest stands (B). LT: on top of the litter; ST: pressed half-way into the soil; LS: beneath the litter of 4cm depth; S1: under 1cm soil without litter cover; LS1: under 1cm soil with 4 cm litter coverage. Seeds in both LT and ST position was not covered by soil or litter, thus the light and temperature conditions should be no different. Data in the same column with different lower case letters represent significant differences between seed positions in the same shading degree (P < 0.05). Data with different capital letters represent significant differences between shading levels in the same seed position (P < 0.05)

Shading degree	Seeding position	PPFD (μmol m^{-2} s^{-1})	R/FR ratio	Temperature (°C)	Relative humidity (%)
A. In shade houses					
0%	LT(ST)	693.27 ± 136.37bC	1.09 ± 0.01bA	24.75 ± 0.53 aB	71 ± 3.8 a
	LS	33.28 ± 5.25 aC	0.75 ± 0.02 aB	25.33 ± 0.68 aC	
	S1	18.48 ± 2.77 aC	0.77 ± 0.02 aA	25.17 ± 0.89 aC	
	LS1	16.63 ± 2.63 aC	0.73 ± 0.03 aA	23.5 ± 1.03 aC	
40%	LT(ST)	402.6 ± 80.83bC	1.09 ± 0.01 bA	23.17 ± 0.49 aB	62 ± 9.3 a
	LS	18.29 ± 3.38aBC	0.77 ± 0.00 aB	23.25 ± 0.31aBC	
	S1	10.10 ± 1.64aBC	0.75 ± 0.03 aA	22.92 ± 0.44 aBC	
	LS1	9.61 ± 1.77 aBC	0.75 ± 0.02 aA	22.00 ± 0.68 aBC	
70%	LT(ST)	179.81 ± 32.13bB	1.07 ± 0.01 bA	20.75 ± 0.51aA	69 ± 10.5 a
	LS	9.63 ± 2.04 aB	0.79 ± 0.04 aB	21.83 ± 0.48 aAB	
	S1	6.84 ± 1.42 aB	0.76 ± 0.03 aA	21.67 ± 0.59 aAB	
	LS1	5.02 ± 0.92 aB	0.75 ± 0.03 aA	20.50 ± 0.57 aAB	
85%	LT(ST)	97.36 ± 19.95 cAB	1.05 ± 0.03 bA	20.33 ± 0.53 aA	80 ± 4.8 a
	LS	4.43 ± 0.82 bA	0.78 ± 0.01 aB	20.75 ± 0.51aA	
	S1	2.64 ± 0.46abA	0.77 ± 0.02 aA	20.75 ± 0.58 aA	
	LS1	2.22 ± 0.35 aA	0.78 ± 0.02 aA	19.67 ± 0.53 aA	
95%	LT(ST)	59.70 ± 13.53 bA	1.04 ± 0.01 bA	19.92 ± 0.24 aA	79 ± 11 a
	LS	4.01 ± 0.85 aA	0.68 ± 0.02 aA	20.5 ± 0.22 aA	
	S1	2.44 ± 0.52 aA	0.68 ± 0.03 aA	19.83 ± 0.31aA	
	LS1	2.28 ± 0.52 aA	0.68 ± 0.03 aA	19.17 ± 0.41aA	
B. In forest stands					
85%	LT(ST)	136.25 ± 49.83bB	0.73 ± 0.03bB	19.63 ± 0.4aA	85 ± 4.5 a
	LS	4.49 ± 1.38aB	0.51 ± 0.02aB	20.11 ± 0.34aA	
	S1	3.44 ± 1.02aB	0.52 ± 0.03aB	19.78 ± 0.4aA	
	LS1	2.92 ± 0.86aB	0.51 ± 0.03aB	19.18 ± 0.51aA	
90%	LT(ST)	36.07 ± 8.81bA	0.56 ± 0.03bB	18.9 ± 0.41aA	64 ± 7.4 a
	LS	1.63 ± 0.39aA	0.38 ± 0.03aAB	19.37 ± 0.35aA	
	S1	1.11 ± 0.27aA	0.39 ± 0.03aAB	19.13 ± 0.39aA	
	LS1	0.94 ± 0.22aA	0.36 ± 0.02aA	18.53 ± 0.44aA	
95%	LT(ST)	8.70 ± 1.62bA	0.29 ± 0.01aA	18.93 ± 0.51aA	85 ± 2.1 a
	LS	0.76 ± 0.13aA	0.22 ± 0.01aA	19.33 ± 0.39aA	
	S1	0.50 ± 0.10aA	0.21 ± 0.01aA	19.17 ± 0.62aA	
	LS1	0.44 ± 0.03aA	0.20 ± 0.01aA	18.6 ± 0.47aA	

Data analysis

The environmental characteristics (light, temperature and relative humidity) under different shading and seed positions were compared using one-way ANOVA analysis and Fisher's LSD test. The relationship between light intensity (PPFD) and light quality (R/FR) was estimated using a power function curve fit.

Both the final seed germination percentage and germination process were employed to explain the characteristics of seed germination responding to different treatments. The germination percentage —with its process expressed as $t_{50\%}$, and showing the number of days to complete 50% germination— was calculated as the percent of the number of germinated seeds accounting for the total number of sown seeds. When necessary, and using normal probability plots, all data were tested for normality and logarithmic, or arcsine-square-root, transformations. The effects of shading, seed position and two-way interactions (i.e., shading by seed positions) on seed germination (including germination percentage and process) in shade houses were examined with UNIAN-

OVA analysis. Fisher's LSD test was applied *post hoc* to distinguish the germination among the treatments. The exponential function was applied to analyze the relationship between $t_{50\%}$ and shading degree for seeds in five positions. Only the significant relationships for seeds in LT/ST were presented in the present study.

Germination fluctuated in forests after leaf expansion but did so much less than in the shade houses, as the germination data were not normal. Thus, the Kruskal-Wallis test (K-W test) was used to estimate the differences among shading and seed position treatments. Differences at a level of $P < 0.05$ were considered significant. All of the statistical analyses were performed with SPSS software (16th edition, Chicago, USA).

Results

Light and temperature conditions relating to shading and seed position

With the increase of shading degree and seed depth in the soil, the light intensity decreased dramatically, from 693.27 ± 136.37 μmol m^{-2} s^{-1} at 0% shading degree in shade houses to 0.44 ± 0.03 μmol m^{-2} s^{-1} at 95% shading degree in forest stands (Table 1). Only 4.54%-6.72%, 2.51%-4.09% and 2.28%-3.82% of light irradiance could transmit through 4 cm of leaf litter, 1 cm of soil and the combination of both, respectively. Light quality (R/FR) did not change significantly along the shading degrees (from 1.09 ± 0.01 to 1.04 ± 0.01) in shade houses (Table 1A). However, seed positions had a significant effect on light quality in each shading degree. In contrast, light quality in forests with leaf canopy varied with the canopy openness (Table 1B). The temperature ranged from 24.75 to 19.92°C with increasing shading degrees but fluctuated insignificantly among seed positions (Table 1). Although the coverage of litter/soil distinctly reduced light transmittance, no more than 1.2°C variation in temperature was observed between LS/ S1/LS1 and LT /ST (Table 1).

Light quality did not vary with light intensity (PPFD) in shade houses (Fig. 2A). A logarithmic function (Fig. 2B; $F_{1,60} = 108.29$, $P < 0.001$) described the relationship between light quality and light intensity, and no significant differences were found in relative air humidity among all the shading degrees (Table 1).

Seed germination in shade houses

Germination percentage was significantly affected by the shading degree (Table 2A; $P < 0.001$). For seeds sown on top of the litter or soil (LT/ST), the highest germination percentages were observed at a 70% shading degree. Seeds covered by 1 cm depth of soil without litter coverage (S1) reached the maximum germination percentage in 40% shading degree. For seeds in LS and LS1 categories, the highest germination per-

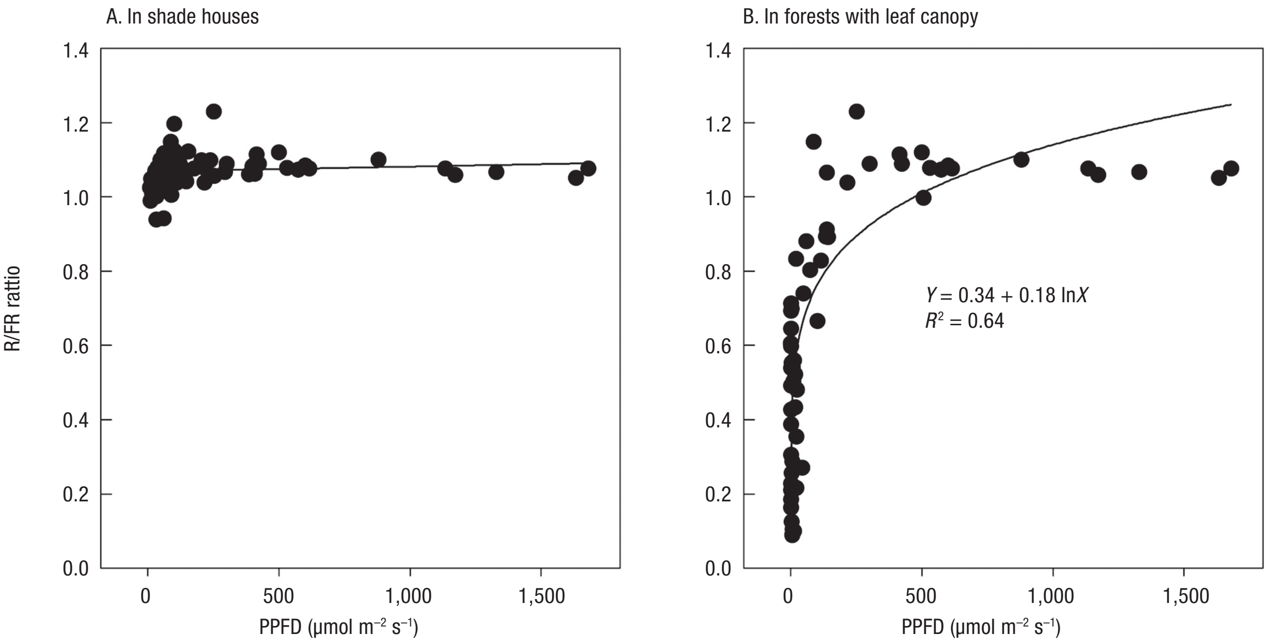

Figure 2. Relationship between light intensity (PPFD) and light quality (R/FR) in shade houses (A) and forests with leaf canopy (B).

Table 2. UNIANOVA on seed germination, at five shading degrees (0%, 40%, 70%, 85%, and 95%) and five seed positions (on top of the litter, pressed half-way into the soil, beneath the litter of 4 cm depth, under 1 cm soil without litter cover, under 1 cm soil with 4 cm litter coverage) in shade houses (A), and at four shading degrees (0%, 55%, 69%, 76%) and five sowing positions in forest stands before leaf expansion (B)

Source of Variation	Germination percentage (%)			Germination process ($t_{50\%}$, days)	
	df	F	P	F	P
A. In shade houses					
Shade degree	4	15.24	< 0.001	6.38	< 0.001
Position	4	23.76	< 0.001	30.21	< 0.001
Shade degree × Position	16	8.33	< 0.001	6.30	< 0.001
B. In forests – before leaf expansion					
Shade degree	3	75.645	< 0.001	–	–
Position	4	45.694	< 0.001	–	–
Shade degree × Position	12	28.398	< 0.001	–	–

centage appeared in full light. The lowest germination percentages for seeds in LT, ST, LS, S1 and LS1 were seen at 0%, 0%, 85%, 95% and 95% shading degrees, respectively (Fig. 3).

The value of $t_{50\%}$ also differed among the observed shading treatments (Table 2A; $P < 0.001$). For seeds in LT/ST, $t_{50\%}$ decreased regularly with the increase of shading degrees (Fig. 4A). The power function was found to describe strong correlations between shading degree and $t_{50\%}$ only for seeds on top of the litter/soil (LT/ST, Fig. 4B). However, for seeds in LS/S1/LS1,

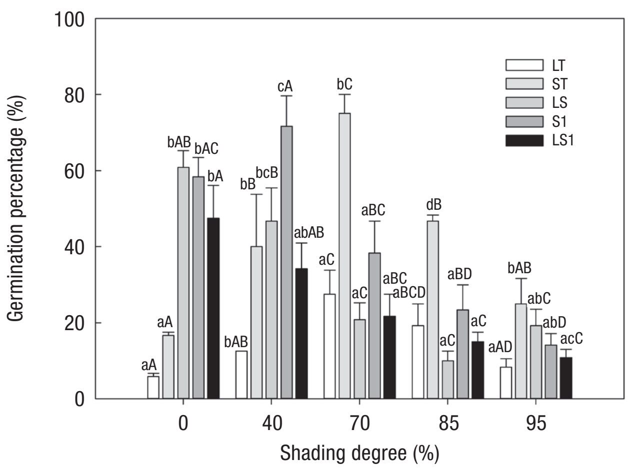

Figure 3. Germination percentages among different shade and seed position treatments in shade houses. Bars are Means ± SE. Bars with different lowercase letters represent significant differenced among seed position treatments in the same shading degree ($P < 0.05$). Bars with different capital letters represent significant differenced among shading levels in the same seed position ($P < 0.05$).

$t_{50\%}$ remained similarly low regardless of the increase of the shading degree.

UNIANOVA indicated that interactions of shading degrees by seed positions on both germination percentage and process were significant ($P < 0.05$) (Table 2A).

Seed germination in forests

The shading degrees in the forests changed from 55% to 85%, 69% to 90%, and 76% to 95% with the expansion of leaf canopy. Therefore, in addition to full light treatments, seven shading degrees were obtained in the forests throughout the whole growing season. The germination percentage in forests, before or after leaf expansion, was significantly different across the seven shading degrees and five seed position treatments (Tables 2 and 3, Fig. 5). Before leaf expansion, the germination percentage reached 80% for seeds in 76% shading degree without litter/soil coverage. Conversely, germination after leaf expansion could only achieve at most 20% until the end of the growing season (Fig. 5). The germination percentage for seeds in ST was almost the highest of all the five seed positions along the shading degrees, ranging from 55% to 95% (Fig. 5). For seeds in LT/ST/LS/S1, the 76% shading degree favored the highest germination percentage among all the treatments (Fig. 5). Except for the full light treatment, the lowest germination percentages were recorded in LS1 for the other six shading degrees (Fig. 5). At 55% and 95% shading degrees, seeds germinated significantly less in LS/S1/LS1 than those in ST. After leaf expan-

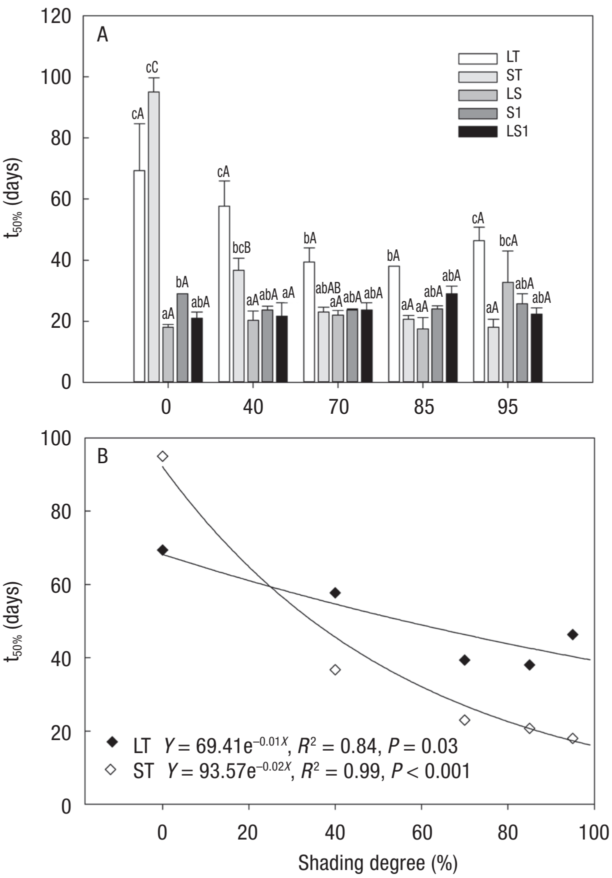

Figure 4. $t_{50\%}$ under various shading and seed position treatments in shade houses (A), and the relationship between shading degree and $t_{50\%}$ for seeds sowing on top of the litter (LT) and of the soil (ST) (B). Data was based on $t_{50\%}$ in LT and ST along the shading levels in shade houses. Bars are means ± SE. Bars with different lowercase letters represent significant differences among seed positions in the same shading degree ($P < 0.05$). Bars with different capital letters represent significant differences among shading levels in the same seed position ($P < 0.05$).

sion, a significant effect of shading on the germination percentage was only found for seeds in ST (Table 3). For seeds in ST, an 85% shading degree favored the highest germination percentage (Table 3). For seeds in LT/LS/S1/LS1, no significant differences of germination percentage were found among the shading degrees.

Discussion

Distinct germination percentages under leaf canopy, leafless canopy and in shade houses

Overall, the results indicated that the germination percentage was distinct among forest stands under leaf canopy and leafless canopy, as well as in shade houses. The minority of seeds (≤ 20%) germinated under leaf canopy, while the majority (nearly 80%) germinated under leafless canopy for seeds not covered by litter. This finding indicates that for *P. koraiensis,* an important germination strategy can be implemented through the enlargement of canopy openness during early spring. The decreases in germination percentage under leaf canopy should be due to the comprehensive effects of light reduction caused by the increase of shading degrees, from 55% to 85%, 69% to 90% and 76% to 95%, resulting from leaf expansion (Table 1). Furthermore, even in the same shading degree (85% and 95%, with the same light intensity) for the seeds without coverage (LT and ST), the germination percentage under leaf canopy was significantly lower than that in shade houses (in an independent t test, all $P < 0.05$; see Figs. 3 and 5B). The cause of this discrepancy might be due to the differences of light quality induced by nylon nets and leaves. Light transmitted through nylon nets (similar to tree trunks and branches), reduced light intensity without changes in light quality (Fig. 2A). In contrast, and in accord with the results from the hard forest in Japan (Seiwa *et al.*, 2009), we found that light quality co-varied with light irradiance (intensity), following the logarithm function (Fig. 2B) under leaf canopy. Moreover, this effect of light quality on *P. koraiensis* seed germination was confirmed by the greater germination percentage found under a higher R/FR ratio in growth chambers (unpublished data).

From 85% to 95% shading degree, the responses of seeds in the same position to the shading degrees were different between forest stands and shade houses. In shade houses, no significant differences of germination percentage were found in this range regardless of the seed positions. Nevertheless, it was found that for seeds in forest stands and in ST, the 85% shading degree favored higher germination percentages than the 95% shading degree (Fig. 5). This result indicated that seeds could utilize enhancive light irradiance results from the increase in canopy openness when soil or litter did not cover them. However, seeds covered by litter or by the combination of litter and soil were barely sensitive to the alteration of the shading degree from 85% to 95% (Fig. 5B). This result suggested that by removing the litter coverage for seeds on top of the soil (ST), *P. koraiensis* germination would increase by utilizing the formation of a gap. Castro *et al.* (2002) reported a similar result for *P. sylvestris* seeds sown on the soil surface, which emerged low from undisturbed coverage

Table 3. Kruskal-Wallis test on seed germination percentages, at three shading degrees (85%, 90%, 95%) and five seed positions (on top of the litter (LT), pressed half-way into the soil (ST), beneath the litter of 4 cm depth (LS), under 1 cm soil without litter cover (S1), under 1 cm soil with 4 cm litter coverage (LS1)) in forests after leaf expansion. Differences at a level of Sig. (χ^2) < 0.05 were considered significant

Shading degree	**Seed position**					**Sig. (χ^2)**
	LT	**ST**	**LS**	**S1**	**LS1**	
85%	5.83	13.33	3.33	8.33	1.67	0.04
90%	5.00	6.67	1.67	4.17	1.67	0.23
95%	1.67	5.00	2.92	1.67	1.67	0.02
Sig. (χ^2)	0.32	0.047	0.36	0.18	0.96	–

and high when the coverage of herbaceous layer was removed.

Similar germination percentages under leafless canopy and in shade houses

Nylon shading, which produced similar light conditions to those of natural forests during early spring, made it feasible to control the shade and seed positioning across the entire germination process. In early spring before leaf expansion, reduced light levels reaching the forest floor do not follow coinstantaneous changes of light quality (Smith, 1982) because the tree trunks and branches are the main shade elements. Shade houses established by nylon nets not only created similar light intensity but also similar light quality, including changes of sun flecks under leafless canopy. Similar response patterns of seed germination were found in shade houses and in leafless forests (Fig. 3 and 5), further demonstrating that the use of neutral cloth as shade housing provides a feasible way to simulate the leafless canopy in early spring.

Overall, the highest germination percentage (ca. 80%) appeared at similar shading degrees; that is, at a 70% shading degree (seeds in ST) in shade houses, and at a 76% shading degree (seeds in ST/S1) in forests before leaf expansion. This result may suggest that ca.

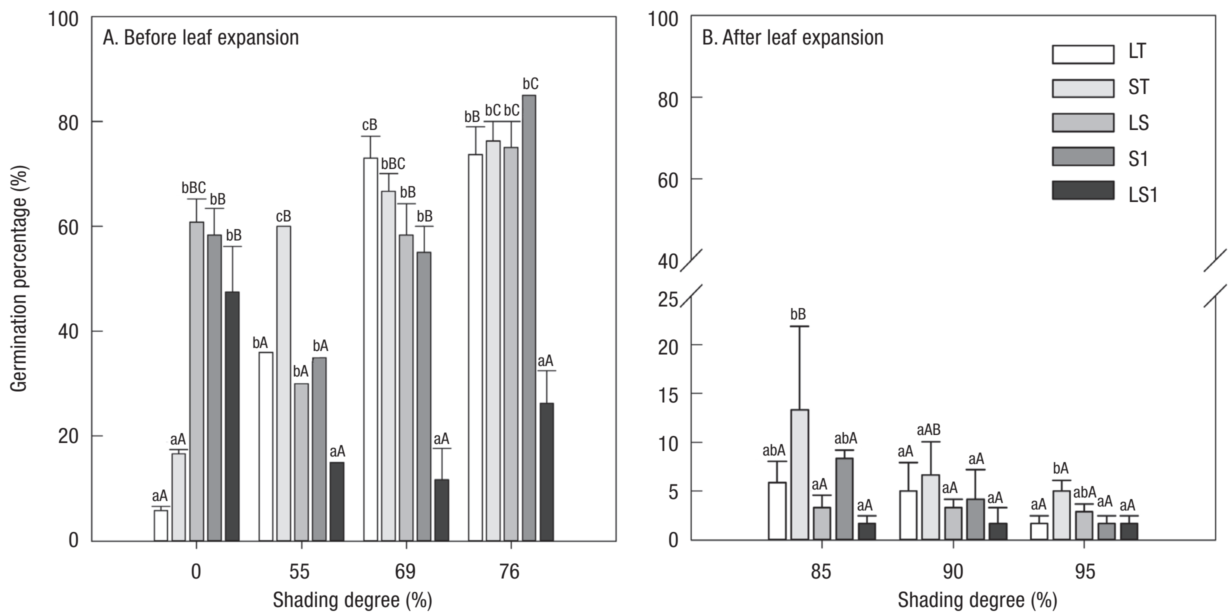

Figure 5. Germination percentages among different shade and seed position treatments in natural forests before leaf expansion (A) and after leaf expansion (B). Bars are Means ± SE. Bars with different lowercase letters represent significant differences among seed positions in the same shading degree (P < 0.05). Bars with different capital letters represent significant differences among shading levels in the same seed position (P < 0.05).

70% shading degree without litter or soil coverage provided the most efficient environmental conditions for *P. koraiensis* germination. This observation was especially true for 70% to 95% shading degree in shade houses, where seeds likely did not suffer stresses from moisture or temperature (Table 1). The decrease in germination percentage demonstrated the positive impact of light irradiance on *P. koraiensis* germination. Similarly, Ruano *et al.* (2009) found that the amount of light affected the germination of *P. pinaster*, where a lower germination rate occurred in areas without basal area removal than in sites with 25% basal area removal. The temperatures in the small gaps, especially for seeds on the soil surface, did not differ greatly from that in the understory. Therefore, in these microsites, irradiance would be an important factor for the germination of light-demanding species (Pearson *et al.*, 2002). Although it was proposed that light sensitivity of seed germination is progressively reduced with the increase of seed mass (0.0320-22.2000 mg) (Milberg *et al.*, 2000), *P. koraiensis* seeds of large size (500-600 mg) exhibited light sensitivity in this study. In our research, the effect of light on the seed germination of *P. koraiensis* was consistent with many other *Pinus* species. Generally, the light requirements and properties of phytochrome for seed germination were frequently found in many species of *Pinus* spp. (e.g., *Pinus taeda* L., *P. strobus*, *P. sylvestris*, *Pinus halepensis* Mill., *Pinus brutia* Ten., *Pinus elliottii* Engelm.) (Toole *et al.*, 1962; Grill and Spruit, 1972; Thanos and Skordilis, 1987; Burgin *et al.*, 1999). Light requirements for seed germination of *P. koraiensis* imply that proper harvest intensity should be applied to improve the regeneration of this species.

Similarly, the germination percentage for seeds not covered by litter/soil decreased with the alteration of shading degree from 70% to 40% in shade houses, and from 70% to 55% in forests before leaf expansion. These observations indicated that higher light irradiance created by 40% or 55% shading might not be beneficial to *P. koraiensis* seed germination. Moreover, the high germination percentage (47%-72%) for seeds beneath the litter/soil at 0% and 40% shading degrees illustrated that low light irradiance (10.10-33.28 μmol m^{-2} s^{-1}, ca. 1.5%-4.8% light irradiance) could initialize the germination of *P. koraiensis*. The low seed germination percentage in full light (0% shading degree) with seeds on top of the soil (16.17%) was partly attributed to desiccation caused by high irradiance (Kyereh *et al.*, 1999; Makana and Thomas, 2005). The lowest germination percentage in full light for seeds on top of the litter (5.83%) might be due to the physical barrier effect of litter between the seeds and soil (Molofsky and Augspurger, 1992; Vázquez-Yanes and Orozco-Segovia, 1992).

In each shading degree, the germination pattern in response to seed positioning was also almost the same between shade houses and leafless forests: germination was inhibited for seeds in LS/S1/LS1 when the germination percentage was lower than that in ST. The results from both shade houses and leafless forests demonstrated that the germination of seeds covered by litter and soil was the most hindered among all the positions as the shading degree became larger than 40%. The main reason for these differences was the variations in the microenvironment (e.g., light, temperature) dictated by the seed position (Pons, 1992; Farrell *et al.*, 2011). As shown in Table 1, the temperature fluctuation among seed positions at each shading degree was subtle, while the light conditions were greatly changed with the modification of seed position. Hence, the drastic changes in the light regimes caused by various seed positions contributed substantially to the seed germination patterns in different seed positions.

The ecological significance of light requirement for *P. koraiensis*

Grill and Spruit (1972) validated light mediation in the germination of *Pinus* spp. seeds. The germination percentage of the second most common, five-leaved species *P. strobus*, which has smaller seeds (13-26 mg) than *P. koraiensis*, also exhibited obvious discrepancies under various light qualities (Toole *et al.*, 1962) and intensities (Parker *et al.*, 2006). Knowledge of the effect of light irradiance on the germination of *Pinus sibirica* (Loud.) Mayr., the closest sibship with *P. koraiensis*, was unavailable. Both *P. sibirica* and *P. koraiensis* are characterized by a thick opaque seed coat and large seed mass (500-600 mg). According to Millberg *et al.* (2000), large seeds (maximum seed mass: 22 mg) seem to be less dependent on light irradiance. However, Mamo *et al.* (2006) found no strong correlation between seed morphological traits and germination in light, darkness or nursery beds for *Juniper* seeds. Yirdaw and Leinonen (2002) reported the significant effect of light quality on seed germination of *Cordia Africana* Lam. (median seed mass: 214 mg), which elucidated that seed morphological traits might not be an adaptive indicator in predicting the light requirement of seed germination. It was also reported that the mechanical restraint of the thick seed coat was diminished as the *P. sibirica* embryo became truly mature by undergoing natural cold stratification from winter to early spring (Tillman-Sutela *et al.*, 2008), which was also true for *P. koraiensis.* The light requirement for the germination of *P. koraiensis* might be created after seed prechilling, as has been reported for *P. strobus* (Toole *et al.*, 1962). Mature seeds of *P. koraiensis* initiate germination by perceiving light irradiance through the phytochrome. To better elucidate the mechanisms of phytochrome mediation by prechilled *P. koraiensis* seeds, further study should be focused on the physiological changes of these large seeds under various light irradiances.

The light requirements for the germination of *P. koraiensis* have significant implications to ensure the regeneration of the *P. koraiensis* population in temperate secondary forests. Pine seeds germinated under suitable microclimates through perceived light alterations would escape rodents, which would guarantee the seedling survival of the subsequent generation. Tree species with small seed sizes produce large seed numbers, which would increase the probability for seeds to fall and grow in suitable microsites (Pearson *et al.*, 2002). However, as seeds of *P. koraiensis* are relatively larger in seed size and smaller in seed number, they are mostly dispersed by animal cache. Furthermore, the habitat selection for potential seed dispersers to cache the pine seeds is biased to closed and shading forests (Hutchins *et al.*, 1996). All of these traits collectively mean that it is less likely for *P. koraiensis* seeds to reach a suitable gap site. During early spring, and before leaf expansion, there is enhanced and stable light irradiance in deciduous mixed forests. Initiating germination in early spring prior to leaf expansion accomplishes the recruitment of seedlings, as well as offsets the poor mobility of pine seeds. The emerged seedlings in early spring would also store sufficient carbohydrates through photosynthesis to endure the long periods of reduced light resulting from leaf expansion.

Conclusions

It is clear that light conditions affect seed germination of *P. koraiensis*. Early spring prior to leaf expansion offers an alternative timing for seed germination of *P. koraiensis*, as the light irradiance is nearly identical to that of gaps after leaf expansion. Similar germination patterns found between shade houses and leafless forests demonstrate that shade house experiments provided a feasible method to simulate seed germination in early spring. The optimum light condition for the germination of *P. koraiensis* seeds pressed half-way into the soil (ST) was a 70% shading degree in early spring under a leafless canopy. Due to the decline of both light intensity and light quality caused by leaf expansion, the germination percentage was substantially lower in forests after the canopy completely closed. In the common shading degrees (55%-95%) in forests, the germination of *P. koraiensis* seeds beneath litter, soil or the combination of both was strikingly hindered. Thus, appropriate silviculture regulation (e.g., harvest intensity, litter removal) to create more favorable light regimes for germination should be adopted to improve the regeneration of *P. koraiensis*.

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