

Effects of Experimental Early Canopy Closure on the Growth and Reproduction of Spring Ephemeral *Erythronium japonicum* in a Montane Deciduous Forest

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Abstract Spring leaf flush and changes in the understory radiation in montane deciduous forests are major determinants of the life cycle of spring ephemerals, which complete their epigeous growth before canopy closure in spring. We compared the growth, carbon allocation, and reproduction of a spring ephemeral, *Erythronium japonicum* (Balrer) Decne., between the ambient (control) and early shade treatments in the field during the flowering (early-May), fruiting (mid-May), and fruit ripening periods (late-May), under the assumption of early spring canopy closure due to climate change. Carbon allocation was investigated using a ¹³C labelling experiment. Both sterile (non-flowering) and fertile (flowering) *E. japonicum* under the shade treatment showed higher specific leaf area and earlier leaf senescence than those in the ambient conditions ($p < 0.05$). The flowering *E. japonicum* concentrated on biomass allocation to the aboveground vegetative organs prior to fruiting and to reproductive and storage organs from fruiting. *E. japonicum* used carbohydrates stored during the previous year for vegetative growth, while the current-year photosynthates were used for the current-year reproduction. Carbon allocation to fruit began earlier under the shade treatment, demonstrating that *E. japonicum* allocated the current-year photosynthates more to reproduction than to vegetative organs under the early shade conditions. However, the seed size ($p = 0.012$) and germination rate ($p = 0.008$) were significantly lower under the shade treatment than under the ambient conditions, implying a potential decrease in viable seed production in the shorter high-light period. The earlier leaf flush could be a critical threat to the population maintenance of spring ephemerals such as *E. japonicum*.

Keywords: Leaf flushing, Organ biomass, Photosynthate allocation, Reproduction, Resource trade-off, Shading

Introduction

In montane deciduous forests, spring leaf flush and leaf growth proceed within a short period, rapidly closing the overstory canopy and reducing the understory light input. Fast changes in the understory light conditions in spring affect the life cycle of spring ephemerals that appear aboveground in the early spring when the snow melts, quickly bloom, and complete their aboveground life cycle during the high-light period before canopy closure occurs (Schemske et al. 1978; Lubbers and Christensen 1986).

The life cycle of spring ephemerals is closely related to the leaf phenology, which is sensitive to temperature (Vitasse et al. 2009). Climate change is believed to induce changes in spring temperature and leaf phenology (Fitter and Fitter 2002; Menzel et al. 2006). The timing of leaf unfolding in Europe has advanced an average of 6.3 d compared to 30 years ago (Menzel 2000) and *Populus tremuloides* Michaux in western Canada has experienced a shift to blooming 26 d earlier over the last century (Beaubien and Freeland 2000). Studies on plant phenology in a temperate deciduous forest in Gwangneung, Korea, have reported that the leaf-out of *Carpinus laxiflora* (Siebold et Zucc.) Blume and *Quercus serrata* Murray in 2011 was 8–15 d earlier than that in 2010 (Korea National Arboretum 2011). However, the onset of leaf-out still shows substantial annual variations depending on the spring temperature each year. High variations in the overstory phenology resulted in annual changes in the high-light period for spring ephemerals, which affected their growth and reproduction (Nishikawa 2009).

Erythronium japonicum (Balrer) Decne. is a representative

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spring ephemeral in montane forests in Korea. *E. japonicum* is a perennial species with only 3–5 weeks of epigeous growth period. The new shoot of *E. japonicum* is formed within the bulb in late autumn, and remains underground through winter (Kawano 2005). In early spring, the shoot begins to expand and appear aboveground. It finishes the epigeous growth stage before full canopy closure and stays dormant in summer. In autumn, the sprouting of new roots takes place followed by the formation of new shoots. While winter chilling is indispensable for the dormancy release of *E. japonicum* (Kim et al. 2014), *E. japonicum* cultivated at 20°C showed significantly shorter leaf longevity (Yoshie and Fukuda 1994), indicating that this species adapts to cool temperature, rather avoiding summer.

Perennial herbs have a resource trade-off strategy between the current year's reproduction and the next year's growth and reproduction, which varies depending on the species and environmental conditions (García and Ehrlén 2002). Spring ephemerals have wide overlapping periods of vegetative growth and reproduction, making the resource trade-off strategy an important life cycle strategy (Lubbers and Lechowicz 1989; McKenna and Houle 2000). Most of the carbohydrate accumulation of spring ephemerals occurs in the high-light period before the overstory leaf-out in early spring (Risser and Cottam 1967; Sparling 1967). Because the leaves of spring ephemerals senesce before full canopy closure, they hardly acclimate to low radiation (Eickmeier and Schussler 1993). Earlier leaf flushing shortens the high-light period and could reduce carbon gain during the epigeous growing season, accompanying disadvantage for seed production (Sunmonu and Kudo 2014). It is important to understand the effects of the radiation changes in the understory on the resource allocation patterns in order to understand the life cycle strategy of spring ephemerals.

In this study, we compared the growth and reproduction of *E. japonicum* between the ambient and experimental early shade conditions in order to understand the effects of early canopy closure on the growth and reproduction of this perennial spring ephemeral which rarely reproduces vegetatively.

This study assumed that climate change would induce earlier leaf flushing, resulting in shorter high-light period. We hypothesized that earlier leaf flushing would negatively affect *E. japonicum* populations. We (1) examined the environmental characteristics in the ambient and early shade treatment sites, (2) measured and compared the stem and leaf growth, organ biomass, carbon allocation for the current year reproduction and next year's growth, between the ambient and shade treatment sites, (3) investigated how early shading treatment affected the growth and reproduction of *E. japonicum*, and (4) studied the effects of early canopy closure on the *E. japonicum* populations. We expect that the results will be

used to develop *in-situ* conservation measures for perennial spring ephemerals.

Results

Atmospheric, Soil, and Light Conditions

The mean soil temperature from 5 May to 15 June 2013 was $10.5 \pm 0.4^\circ\text{C}$ in the shade treatments, which was 1.1°C lower than that of $11.6 \pm 0.3^\circ\text{C}$ in the ambient conditions ($p < 0.05$; Fig. 1A). The soil water content was ranged in $0.17\text{--}0.32 \text{ m}^3 \text{ m}^{-3}$. The air temperature and soil moisture content from 5 May to 15 June 2013 were not significantly different between the ambient and shade treatments. The study sites were located on steep slopes with rough topography, resulting in high variations in soil moisture content within a short distance. So we focused on the effects of light changes, assuming high variations in soil moisture content regardless of light conditions.

The daily mean photosynthetically active radiation (PAR) and leaf area index (LAI) showed opposite patterns. The

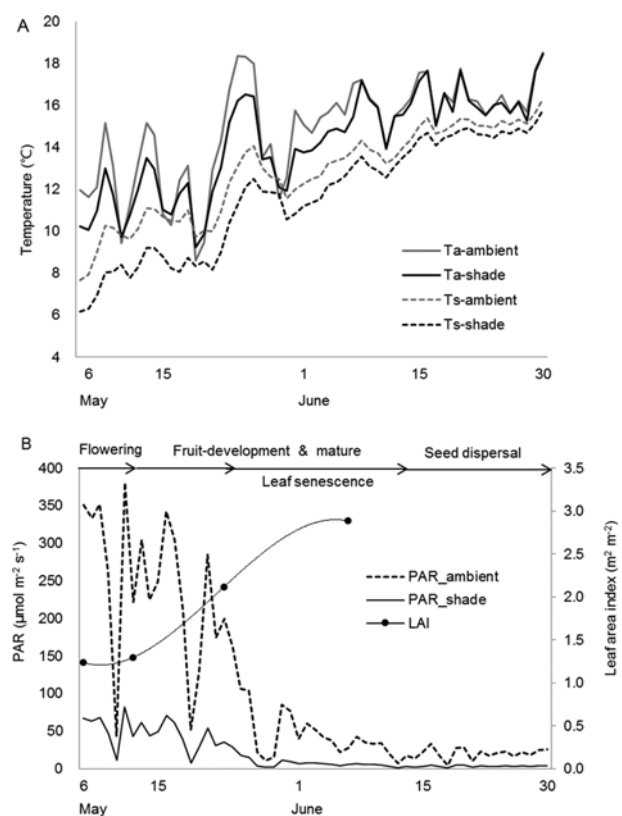


Fig. 1. (A) Mean daily air temperature (Ta) and soil temperature (Ts) and (B) mean daily photosynthetically active radiation (PAR) and leaf area index (LAI) from May 5, 2013 to June 2013 under the ambient and shade conditions. Shading treatment and temperature measurement at the shade treatment sites began on May 5, 2013. Flowering, fruiting, and seed dispersal periods of *E. japonicum* in the study sites are indicated at the top of Fig. 1(B).

daily mean PAR began to decrease noticeably in the ambient plots around May 26, 2013. From May 5 to 26, 2013, the daily mean PAR was $44.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the shade treatment plots, which was less than 20% of the mean PAR of $228.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the ambient plots. Contrary to the PAR, the LAI sharply increased from mid- to late-May and showed similar values in June. The daily mean LAI was $1.23 \text{ m}^2 \text{ m}^{-2}$ on April 26, $1.30 \text{ m}^2 \text{ m}^{-2}$ on May 12, $2.12 \text{ m}^2 \text{ m}^{-2}$ on May 23, and $2.78 \text{ m}^2 \text{ m}^{-2}$ on June 7 (Fig. 1B).

Stem Length and Biomass of *E. japonicum* under the Ambient and Early Shade Treatments

The stem lengths of ambient and shade treatments for both the non-flowering and flowering plants were not significantly different (Table 1). The aerial stem lengths of the non-flowering and flowering plants were 5-7 cm and 13-15 cm, respectively. The underground stem length of the non-flowering plants was 15-20 cm and that of the flowering plants was 14-18 cm in mid-May and late-May.

The non-flowering plants showed significantly greater individual biomass under the ambient conditions than under the shade treatments in both mid-May ($p = 0.014$) and late-May ($p = 0.015$) as the mean individual biomass was 1.29 g and 0.71 g in mid-May, and 1.85 g and 1.30 g in late-May in the ambient and shade treatments, respectively.

The individual biomass of the flowering *E. japonicum* under the shade treatment was not significantly different

from that under the ambient conditions in mid-May. However, it was significantly less in late-May ($p = 0.001$). The mean biomass of the flowering individuals was 2.43 g and 2.05 g in mid-May, and 2.74 g and 1.87 g in late-May under the ambient and shade treatments, respectively.

Leaf Growth and Senescence of *E. japonicum* under the Ambient and Early Shade Treatments

The leaves of *E. japonicum* began to grow in early-May, kept growing until mid-May (around May 14-16), and senesced in late-May. Each leaf of the flowering plants was larger than 30 cm^2 in mid-May.

From early- to mid-May, the mean leaf area growth per flowering individual was 21.4 cm^2 under the shade conditions, which was significantly higher than that of 17.8 cm^2 under the ambient conditions ($p < 0.05$). Both the non-flowering and flowering plants showed greater leaf area growth under the shade conditions than in the ambient conditions during the leaf growth period from early-May to mid-May (Fig. 2).

In mid-May when the leaf area was the largest, the SLA was significantly higher under the shade treatment than under the ambient conditions for both the non-flowering and flowering plants with the mean SLA of $0.26 \text{ cm}^2 \text{ mg}^{-1}$ and $0.34 \text{ cm}^2 \text{ mg}^{-1}$ for the non-flowering plants ($p < 0.001$), and $0.26 \text{ cm}^2 \text{ mg}^{-1}$ and $0.32 \text{ cm}^2 \text{ mg}^{-1}$ for the flowering plants ($p < 0.001$) under the ambient and shade treatments, respectively (Table 1).

Table 1. Stem length (cm), specific leaf area ($\text{cm}^2 \text{ mg}^{-1}$), and individual biomass (g) of the non-flowering (sterile) and flowering (fertile) individuals in mid-May and late-May under the ambient and early shade treatments (Mean \pm SE). The notations *, **, and *** indicate that the means between the ambient and shade treatments in the same row are significantly different at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***) according to an independent *t*-test

Non-flowering	Period	Treatment				t	p
		n	Ambient	n	Early shade		
Aerial stem length (cm)	mid-May	16	6.24 ± 0.37	8	6.63 ± 0.46	-0.63	0.535
	late-May	19	5.77 ± 0.34	9	7.07 ± 0.70	-1.89	0.069
Underground stem length (cm)	mid-May	16	15.36 ± 0.76	8	17.10 ± 1.26	-1.25	0.228
	late-May	19	16.25 ± 0.88	9	18.80 ± 1.15	-1.69	0.103
Specific leaf area ($\text{cm}^2 \text{ mg}^{-1}$)	mid-May	16	0.26 ± 0.01	8	0.34 ± 0.01	-5.41	0.000***
Individual biomass (g)	mid-May	15	1.29 ± 0.13	6	0.71 ± 0.08	2.72	0.014*
	late-May	19	1.85 ± 0.12	9	1.30 ± 0.17	2.62	0.015*
Flowering	Period	n	Ambient	n	Early shade	t	P
Aerial stem length (cm)	mid-May	169	$13.2 \pm 0.34\text{a}$	108	$12.9 \pm 0.48\text{a}$	-	0.972
	late-May	111	$14.7 \pm 0.30\text{b}$	79	$14.8 \pm 0.40\text{b}$	-4.89	0.636
Underground stem length (cm)	mid-May	16	14.9 ± 0.93	7	16.3 ± 1.55	-0.807	0.429
	late-May	21	14.8 ± 0.50	9	15.3 ± 1.16	-0.824	0.417
Specific leaf area ($\text{cm}^2 \text{ mg}^{-1}$)	mid-May	16	0.26 ± 0.01	8	0.32 ± 0.01	4.27	0.000***
Individual biomass (g)	mid-May	15	2.43 ± 0.12	5	2.05 ± 0.31	1.42	0.174
	late-May	20	2.74 ± 0.13	9	1.87 ± 0.16	3.86	0.001**

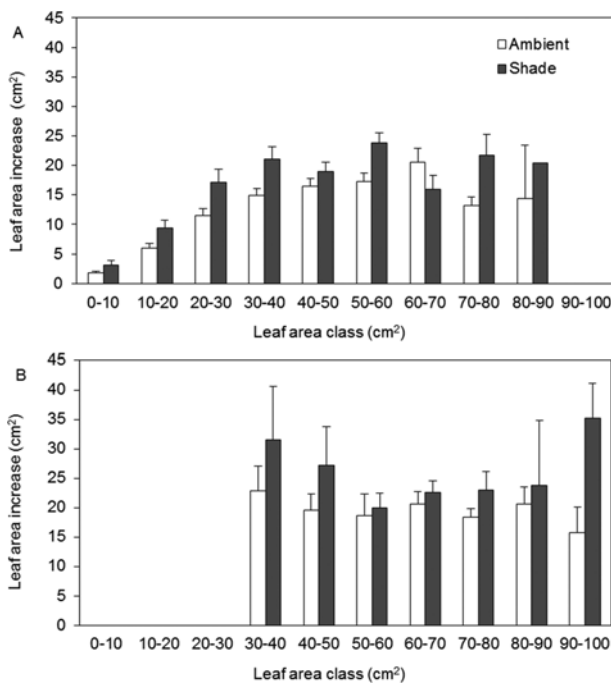


Fig. 2. Mean increase in leaf area (cm²) by individual leaf area (cm²) from early-May to mid-May 2013 for (A) non-flowering and (B) flowering *Erythronium japonicum*. Leaf area class was measured in mid-May 2013. Error bars = SE (n = 2-25). Numbers of leaves measured are 422 (ambient) and 412 (shade) for non-flowering individuals, and 149 (ambient) and 85 (shade treatment) for flowering individuals.

Limited leaf senescence was detected until mid-May. In late-May, the mean ratio of individuals with senescent leaves to the total number of individuals within a plot was 17.4% and 16.5% under the ambient and shade treatments, respectively. The shade treatment plots had significantly higher ratios of dead leaves than the ambient plots in late-May ($p < 0.001$), as the ratio of individuals with dead leaves to the total number of individuals within a plot was 14.0% and 27.1% under the ambient and shade treatments, respectively, indicating that earlier leaf death occurred under the shade treatment (Table 2).

Table 2. Number and ratio (%; data in parenthesis) of fresh, wilting, and dead leaves under the ambient and shade treatments in each period at each study site (18 m²). Measurements were conducted in early-May (May 3-5), mid-May (May 14-16), and late-May (May 24-26). The notation *** indicates that the number of wilting and dead leaves between the ambient and early shade treatments in the same period are significantly different at $p < 0.001$ (***) according to the χ^2 test

Period	Ambient plots			Shade treatment plots			p
	Fresh	Wilting	Dead	Fresh	Wilting	Dead	
early-May	671 (99.9%)	1 (0.1%)	-	601 (99.2%)	2 (0.3%)	3 (0.5%)	0.077
mid-May	678 (98.0%)	12 (1.7%)	2 (0.3%)	613 (98.7%)	4 (0.6%)	4 (0.6%)	0.300
late-May	476 (68.6%)	121 (17.4%)	97 (14.0%)	351 (56.3%)	103 (16.5%)	169 (27.1%)	0.000***

Periodic Changes in the Organ Biomass of *E. japonicum*

The leaf, stem, and bulb biomass of the non-flowering *E. japonicum* individuals in the ambient plots were significantly different between mid-May and late-May, whereas those in the shade treatment plots were not significantly different between mid-May and late-May, except for the bulb biomass (Fig. 3A). The leaf and aerial stem biomass of the non-flowering *E. japonicum* increased from early-May to mid-May, were highest with values of 214.1±16.8 mg and 35.3±3.61 mg in mid-May, and significantly decreased to 149.9±10.7 mg and 22.1±1.9 mg in late-May, respectively, in the ambient plots ($p < 0.05$). On the contrary, the bulb biomass of the non-flowering plants significantly increased from mid-May to late-May in both the ambient and shade treatment plots ($p < 0.01$).

The leaf and stem biomass of the non-flowering *E. japonicum* were not significantly different between the ambient and shade treatments in both mid-May and late-May, while the bulb biomass of the non-flowering individuals in the shade treatment plots was significantly smaller than in the ambient plots in both mid-May and late-May ($p < 0.01$).

Similar to the non-flowering *E. japonicum*, the leaf biomass of the flowering *E. japonicum* increased from early-May to mid-May and significantly decreased from mid-May to late-May under both the ambient and shade treatments (Fig. 3B; $p < 0.001$). Whereas, the fruit biomass significantly increased from 34.7 mg in mid-May to 127.9 mg in late-May in the ambient plots ($p < 0.001$). Therefore, the carbon allocation was concentrated in the vegetative organs for epigeous growth until mid-May and shifted to the reproductive organs after mid-May.

In the flowering individuals, the biomass of each organ was not significantly different between the ambient and shade treatments in mid-May. In late-May, the biomass of each organ of the flowering plants in the ambient plots was significantly greater than in the shade treatment plots, including the fruit ($p < 0.001$), leaf ($p < 0.001$), aerial stem ($p < 0.001$), underground stem ($p < 0.05$), and bulb ($p < 0.01$),

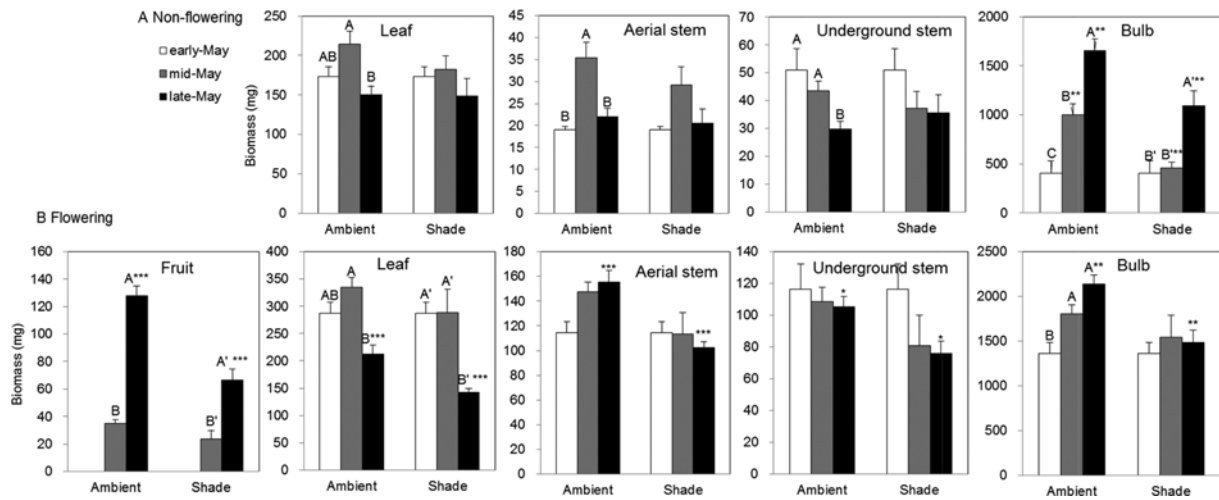


Fig. 3. Organ biomass (mg) by period in the ambient and shade conditions. The values that are followed by the same letter are not significantly different among periods in the same treatment at $p < 0.05$ according to Tukey’s multiple range tests. The Kruskal-Wallis test was conducted to compare leaf and aerial stem biomass of non-flowering plants in the shade treatment and leaf biomass of flowering plants in the ambient condition. The notations *, **, and *** indicate that the means between the ambient and shade conditions in the same period are significantly different at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***). Error bars = SE (n = 3-20).

indicating that the organ growth in the early shade conditions was smaller than that in the ambient conditions.

Current Year Photosynthate Allocation under the Ambient and Early Shade Treatments

The ^{13}C (%) in the aboveground vegetative organs significantly decreased from 37.3% in mid-May to 26.5% in late-May ($p < 0.05$), while that in the belowground organs remained similar between mid-May and late-May under the ambient conditions (Fig. 4A). Under the shade treatment, the ^{13}C (%) in the aboveground vegetative organs significantly decreased between mid-May and late-May ($p < 0.05$), while that in the belowground organs significantly increased from 40.7% in mid-May to 65.4% in late-May (Fig. 4B; $p < 0.05$).

The ^{13}C (%) in the fruit in late-May was 16.6%, which was more than twice as much as 7.7% in mid-May under the ambient conditions, indicating that the photosynthate allocation to fruit in late-May was much higher than that in mid-May. Whereas, the ^{13}C (%) in the fruit in mid-May was 10.4%, which was similar to that of 12.4% in late-May under the shade treatment.

Reproductive Characteristics under the Ambient and Early Shade Treatments

The fruit set in the ambient plot was 76.4% and did not significantly differ from that of 59.7% under the shade treatment (Table 3). The fruit maturity under the ambient conditions was significantly higher than that under the shade treatment ($p = 0.019$). The fruit size was not significantly different between the ambient and shade treatments in mid-

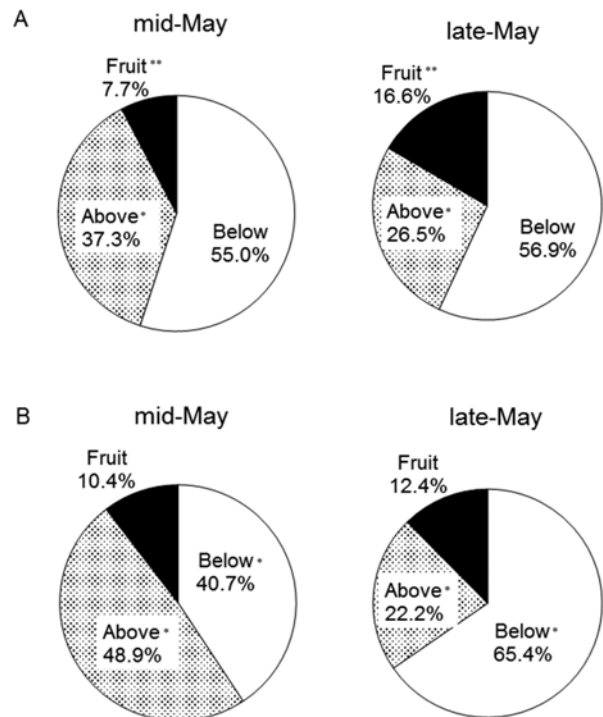


Fig. 4. ^{13}C (%) distribution to fruit, aboveground organs, and belowground organs under the (A) ambient condition and (B) shade condition. The notations * and ** indicate that the means between mid-May and late-May in the same treatment are significantly different at $p < 0.05$ (*) or $p < 0.01$ (**) according to an independent t -test. n = 4 in mid-May and 3 in late-May).

May. However, the ambient plots had significantly greater fruit size with a value of 20.4 cm³ than the shade treatment plots with a value of 12.9 cm³ in late-May ($p = 0.003$).

The shade treatment plots had significantly smaller seed

Table 3. Morphological and reproductive characteristics of the fruit and seed, and the seed germination rate (%) under the ambient and early shade treatments (mean \pm SE). The notations * and ** indicate that the means between the ambient and early shade treatments in the same row are significantly different at $p < 0.05$ (*) or $p < 0.01$ (**), according to an independent *t*-test

Parameter	Treatment				t	p	
	n	Ambient	n	Early shade			
Fruit set (%)	16	76.4 \pm 6.10	14	59.7 \pm 9.61	1.51	0.142	
Fruit mature rate (%)	16	61.9 \pm 6.82	14	37.3 \pm 7.12	2.494	0.019*	
Fruit size (cm ³)	mid-May	15	3.8 \pm 0.41	7	2.8 \pm 0.73	1.18	0.252
	late-May	21	20.4 \pm 1.26	9	12.9 \pm 1.98	3.24	0.003**
Number of mature seeds per fruit	52	27.6 \pm 1.16	41	21.8 \pm 1.21	3.383	0.001**	
Seed size (mm ³)	29	10.4 \pm 0.59	28	8.0 \pm 0.67	2.59	0.012*	
10-seed weight (mg)	30	74.2 \pm 2.01	28	60.9 \pm 3.10	3.65	0.001**	
Mature seed set (%)	52	73.0 \pm 2.15	41	65.6 \pm 2.71	2.17	0.033*	
Seed germination rate (%)	10	76.9 \pm 5.18	7	47.7 \pm 8.80	3.047	0.008**	

size ($p = 0.012$), seed weight ($p = 0.001$), and mature seed set ratio ($p = 0.033$) than the ambient plots. The seed germination rate was also lower in the shade treatments at 47.7%, compared with that of 76.9% in the ambient plots ($p = 0.008$).

Discussion

Effects of Early Shading on the Leaves of *E. japonicum*

The study sites were dominated by deciduous broadleaved trees such as *Q. mongolica* and *Fraxinus* spp., and the canopy leaf flushing began in mid-May, quickly reducing the understory PAR, which coincided with a rapid increase in the atmospheric temperature. The shade treatment in this study imitated earlier leaf flushing and resulted in lower radiation and soil temperature than the ambient conditions. The low radiation reduced the net photosynthetic rates, current-year photosynthate production, and carbon allocation to biomass, resulting in smaller individual biomass under the shade treatment than under the ambient conditions (McKenna and Houle 1999).

The SLA could be interpreted as the leaf area produced by a unit of biomass allocated to leaf production. Shade leaves are generally larger and thinner than sun leaves (Esau 1965). Both the non-flowering and flowering plants had a larger SLA (Table 1) and a higher growth rate in the leaf area (Fig. 2) under the shade treatment than under the ambient conditions, indicating that the leaves of *E. japonicum* could acclimate to the shade conditions and use the limited light resources efficiently by growing into shade leaves (Lambers et al. 2008; Kitaoka et al. 2009). Plants allocate net carbon gain more to leaves and less to roots under shading (Larcher 2003; Park et al. 2010). The increase in the carbon allocation to the leaves expanded the leaf area and could partially

compensate for the low photosynthetic rate and maintain the carbon balance, thus enabling the plants to continue growth under shading (Sparling 1967).

However, *E. japonicum* under the shade treatment showed earlier leaf senescence and leaf death, which was also found in the leaves of *E. americanum* that senesced one week earlier under the closed canopy (Muller 1978). Reduced radiation by canopy closure was a major factor for the leaf senescence of spring ephemerals (Vézina and Grandtner 1965). Species that adapted to intense radiation could maintain high net photosynthetic and growth rates in the high-light conditions, while they showed reduced growth due to a high respiration and light compensation point in the shade (Augsburger 1984). The leaves that adapted to the high irradiance conditions senesced under low irradiance due to an alteration in the chloroplast structure or epigenetic regulations (Lichtenthaler 1981; Humbeck 2013). Earlier senescence of *E. japonicum* under the shade treatment indicated that *E. japonicum* did not acclimate to the earlier canopy closure.

Periodic Changes in the Resource Allocation and Biomass of *E. japonicum*

The temporal changes in the organ biomass accumulation showed the periodic changes in the resource allocation of *E. japonicum* (Fig. 3). Under the ambient conditions, the aboveground vegetative organ biomass increased, peaked in mid-May, and decreased or maintained similar biomass from mid-May to late-May. Whereas, the fruit biomass continued to increase beyond mid-May to late-May during the understory radiation input reduced by the canopy closure, indicating that flowering *E. japonicum* concentrated resource allocation to aboveground vegetative growth until mid-May and to reproduction after that time. Periodic differences in the biomass accumulation between the vegetative organs and the

reproductive organs were also found in *Trillium apetalon* (Ida and Kudo 2008). Non-flowering plants, which did not have reproductive organs, did not switch resources between the current year reproduction and the next year's vegetative growth. The continuous biomass accumulation of the bulb from early-May to late-May could be the result of carbon allocation for the carbohydrate reserve, because bulb growth had occurred in autumn of the previous year (Blodgett 1910; Lapointe 2001).

A comparison of the periodic organ biomass between the ambient and shade treatments showed the source of carbohydrates used for vegetative growth and reproduction (Fig. 3). The flowering *E. japonicum* under the shade treatment had similar biomass in mid-May, however, it had a smaller biomass in late-May than that under the ambient conditions, showing that early shading limited the biomass production after mid-May when the aboveground vegetative growth was almost complete. The similar biomass between the ambient and shade conditions in mid-May indicated that *E. japonicum* used photosynthates stored in the belowground organs during the past year for vegetative growth until mid-May and current year photosynthates for reproduction beyond mid-May. These results were in agreement with the results of previous studies reporting that the vegetative growth and flowering of spring ephemerals depended on the carbohydrates stored in the belowground organs during the previous year (Muller 1978; Routhier and Lapointe 2002). Different sources and the time lag of resource allocation to the vegetative and reproductive organs suggested that the direct trade-offs in the resource allocation between the vegetative organs and reproductive organs were not strong (Sunmonu and Kudo 2014).

Effects of Early Shading on Current Year Photosynthate Allocation

The bulb biomass continuously increased from early-May to late-May, contrary to our expectation of a decrease due to the consumption of stored carbohydrates in the bulb for vegetative growth until mid-May. The ^{13}C ratio showed that the similar ratio of current year photosynthates was translocated to the belowground organs from mid-May to late-May (Fig. 4), indicating that continuous translocation of the current year photosynthates to the bulb, which would probably be allocated as a resource sink rather than for bulb growth (Gandin et al. 2011).

The ^{13}C ratio in the fruit doubled and that in the belowground organs stayed similar from mid-May to late-May in the ambient conditions, indicating that the current year photosynthate allocation to the resource sink was maintained in a similar ratio, while the allocation to reproduction increased after the canopy closure. Contrary to the ambient conditions,

the ^{13}C ratio in the fruit stayed similar from mid-May to late-May in the shade treatment.

The *E. japonicum* plants under the shade treatment showed a lower ^{13}C ratio in the belowground organs and a higher ^{13}C ratio in the fruit compared to those under the ambient conditions in mid-May, indicating more allocation of current photosynthates to fruit and less to belowground organs in mid-May under the early shading conditions. In natural conditions, fruit ripening occurred in the low-light conditions when the canopy closure was already completed, which reduced the photosynthetic production and caused a decrease in the carbohydrate allocation to the roots and an increase in that to the fruit. Plants under the shade conditions allocated resources to reproduction earlier than those under the ambient conditions. All of the organs, except for the fruit, showed a decrease in biomass from mid-May to late-May under the shade conditions, which also showed the priority allocation of resources to reproduction under the shade conditions. Therefore, priority of the photosynthetic allocation was switched from storage to reproduction based on the light conditions (Dale 1992; Horibata et al. 2007).

Effects of Early Shading on Reproduction

The periodic biomass accumulation and ^{13}C ratio in organs showed that *E. japonicum* used current year photosynthates for reproduction. Despite prior resource allocation to reproduction, the fruit biomass, fruit ripening, and viable seed production under the shade conditions were low compared to those under the ambient conditions, implying that the current year photosynthetic production was not enough to develop a fruit, which occupied only 1-4% of the entire plant biomass under the shade conditions (Ghering and Delph 2006). Reduced current year photosynthetic production due to earlier shading reduced resource allocation to reproduction and resulted in a decrease in the seed production in both quantity and quality, and seed germination (Benvenuti et al. 1994). A decrease in viable seed production could accompany a decrease in population, because vegetative reproduction is rare in *E. japonicum*. Therefore, early spring warming could be a critical threat to spring ephemeral populations such as that of *E. japonicum*.

Materials and Methods

Study Sites and Shade Treatment

This study was conducted in *E. japonicum* habitats (37° 28 N, 128° 31 E; 1367 m) on Mt. Joongwang, Korea. This area is a northern temperate zone with four distinct seasons. Meteorological data from the automatic weather station in the study area in 2012-2013 showed that the annual mean air temperature was 5.0°C, with a maximum temperature of 27.7°C in August and a minimum temperature of

Table 4. Topographic and vegetation characteristics of the study sites on Mt. Joongwang, Korea. The two study sites have similar environmental conditions. The height and coverage of the herb layer was measured in early-May (May 3–5), mid-May (May 14–16), and late-May (May 24–26) 2013

		Site 1	Site 2
Latitude		N 37°28'42.9"	N 37°28'43.9"
Longitude		E 128°31'18.0"	E 128°31'15.3"
Altitude (m)		1,195	1,176
Aspect (°)		22.5	22.0
Slope (%)		42	40
Mean height of overstory (m)		10.5 ± 1.2	10.0 ± 0.8
Tree basal area (m ² ha ⁻¹)		25.47	25.48
Mean height of herb layer (cm)	early-May	8.1 ± 0.4	7.5 ± 0.2
	mid-May	14.5 ± 0.5	16.1 ± 0.6
	late-May	20.5 ± 0.9	22.3 ± 0.9
Mean herb coverage (%)	early-May	19.2	20.5
	mid-May	47.1	45.6
	late-May	79.1	65.0

-25.7°C in January. The annual precipitation in 2012–2013 was ca. 1170 mm and over 60% of the precipitation was concentrated between June and September (Korea Meteorological Administration 2014). The snow depth ranged from 35 cm to 60 cm on March 16, 2013, and 22 cm to 37 cm on April 13, 2013. Most of the snow melted before late April on Mt. Joongwang. Soils are acid brown forest soils and classified as Cheongsim series (Loamy skeletal, mesic family of Typic Eutrudepts) (National Academy of Agricultural Science 2011). The bedrock is fine textured sandstone and gray shale materials. The mean soil pH of the study sites was 5.1, the mean soil organic matter content was 18.1%, the mean soil cation exchange capacity was 25.4 cmol kg⁻¹, the mean total N was 0.64%, and the mean available phosphorus was 24.8 mg kg⁻¹.

The overstory was dominated by deciduous broadleaved species including *Quercus mongolica* Fisch. ex Ledeb., *Fraxinus rhynchophylla* Hance, *Acer pictum* subsp. *mono* (Maxim.) Ohashi, *Cornus controversa* Hemsl. ex Prain, *Acer pseudosieboldianum* (Pax) Kom., and *Tilia amurensis* Rupr. The shrub layer was not densely developed and species such as *Philadelphus schrenkii* Rupr., *Staphylea bumalda* DC., *Symplocos chinensis* f. *pilosa* (Nakai) Ohwi, *Deutzia glabrata* Kom., and *Lindera obtusiloba* Blume were distributed in a scattered pattern. Large seasonal differences in the temperature and precipitation induced seasonal differences in the species composition in the understory. The dominant herb species in the spring were *E. japonicum*, *Anemone raddeana* Regel, *Anemone koraiensis* Nakai, *Astilbe rubra* Hook.f. et Thomson, *Pseudostellaria setulosa* Ohwi, *Pimpinella brachycarpa* (Kom.) Nakai, *Corydalis* spp., *Meehania urticifolia* (Miq.) Makino, and *Smilacina japonica* A. Gray.

Two study sites with similar topographic characteristics were selected (Table 4). At each study site, six 3 m × 3 m plots were established with three plots shaded and three plots under ambient conditions. The shade treatment was established with black plastic filters aimed at the simulation of light conditions that were similar to the herb layer under a fully closed canopy. The light intensity in the understory under fully closed canopy conditions in July–September on Mt. Joongwang was 53.7 ± 20.9 μmol m⁻² s⁻¹. A 4 m × 4 m light filter was attached to sticks and placed ca. 1.8 m above the ground for shade treatment. The length of the light filter was 1 m longer than the length of the plot in order to prevent reflected light from entering from the sides. The shade treatment began on 5 May 2013, which was

approximately one week after the blooming of *E. japonicum*, in order to avoid shading effects on the flowering. The 3 m × 3 m plot was divided into nine 1 m × 1 m subplots. Three subplots in each plot were used for periodic growth measurements and the other six subplots were used for a ¹³C labelling experiment and the periodic harvesting of *E. japonicum* for biomass measurements.

Environmental Conditions

HOBO weather stations (HOBO U23 Pro v2, Onset Computer Corporation, Bourne, USA) were placed at plots under both ambient and shade treatments in order to collect data on the air and soil temperature, relative humidity of the air, the soil water content, and the photosynthetically active radiation (PAR, μmol m⁻² s⁻¹) during April–June 2013. Data on the air temperature (°C) and the relative humidity of the air (%) were measured at 1.3 m above the ground. The soil temperature (°C) was measured using a HOBO soil temperature sensor (S-TMB-M002, Onset Computer Corporation) and the soil water content (m³ m⁻³) was measured using a HOBO soil moisture sensor (S-SMB-M005, Onset Computer Corporation) in 30-minute intervals at a 5-cm soil depth.

We took three fish-eye photographs of the canopy at each study site four times from late April to early June 2013 (April 26, May 12, May 23, and June 7) in order to monitor the temporal changes in the canopy closure and the estimated leaf area index (LAI) using a Hemiview software ver. 2.1 (Delta T Devices Ltd., Cambridge, UK). The LAI of the site was calculated by averaging three values of the LAI estimated from the three fish-eye photographs randomly positioned at the study sites.

Growth Measurements

The *E. japonicum* plants emerged in late April when the snow had almost melted away on the forest floor and it bloomed during the first week of May. In mid-May, *E. japonicum* began to produce small fruits and the canopy leaf flushing started. Approximately half of the canopy closure occurred during the fruit ripening of *E. japonicum* that occurred in late May. In early June, the canopy was fully closed, the fruit size of *E. japonicum* increased further, and the leaves of *E. japonicum* were almost gone. Growth measurements were conducted three times at flowering (May 3–5, 2013), fruiting (May 14–16, 2013), and fruit ripening (May 24–26, 2013).

E. japonicum stays sterile (non-flowering) for eight to ten years before being rendered fertile (flowering) by sexual maturity (Yokoi 1976; Kawano et al. 1982). Sterile *E. japonicum* has only a single leaf, while fertile *E. japonicum* has two leaves and a single scape with one flower (Kawano 2005). Measurements of non-flowering and flowering individuals were compared separately, because sterile and fertile *E. japonicum* have differences in age, size, and morphological characteristics.

In each plot, three 1 m × 1 m subplots were randomly chosen and plants < 1 m in height in the subplots were measured. A total of thirty-six 1 m × 1 m subplots were used for the growth measurements: three subplots in each of the three ambient and three shade treatment plots of the two study sites. For all of the *E. japonicum* plants in the three subplots in each plot, we mapped, marked with a numbered tag, and measured the aerial stem length, leaf length and width, leaf damage, flowering, fruiting, and fruit ripening. For the other plants, the species name, height (cm), and coverage (%) were recorded.

E. japonicum was harvested three times: at the flowering (May 5, 2013), fruiting (May 16, 2013), and fruit ripening and leaf senescence (May 26, 2013) periods; harvested materials were moved to the laboratory for dry weight measurements and growth characteristics examination. Considering the results of a previous study reporting the high correlation between the leaf area, age, and biomass (Kawano et al. 1982), we tried to harvest individuals with similar leaf areas for

both the non-flowering and flowering individuals in order to reduce errors caused by age difference. The number of non-flowering plants harvested for biomass were 4 (early-May), 15 (mid-May), and 19 (late-May) in the ambient plots, and 6 (mid-May) and 9 (late-May) in the shade treatment plots. For the biomass measurements of flowering plants, 5 (early-May), 14 (mid-May), and 20 (late-May) plants in the ambient plots, and 5 (mid-May) and 9 (late-May) plants in the shade treatment plots were harvested.

The plants were separated into flower or fruit, leaf, aerial stem, underground stem, and bulb, and the length of each organ was measured. The stem was divided into aerial stem and underground stem, and measured separately to combine the data for aerial stem length measured at the field with those measured in the laboratory. The leaf area was estimated using the correlation equation developed from the leaf length and width measured in the field, and the leaf area measured by a portable leaf area meter (LI-3000C, LI-COR Inc., Lincoln, USA). The leaf biomass (mg) and specific leaf area (SLA; $\text{cm}^2 \text{mg}^{-1}$) of individual plants were measured using the harvested *E. japonicum*. After the measurements, each plant organ was dried at 70°C for 72 hours and the dry weights were measured. The biomass comparison between the ambient and shade conditions was conducted for the *E. japonicum* harvested on May 16 and 26, 2013, because the shade treatment began on May 5, 2013.

¹³C labelling Experiment

¹³C labelling was conducted to elucidate the current year photosynthate allocation for the flowering individuals. The flowering *E. japonicum* individuals were exposed to the ¹³CO₂-enriched conditions in mid-May (May 15-16) and late-May (May 25-26). For the application of ¹³CO₂, each *E. japonicum* individual was enclosed in a 40 cm × 30 cm sealed plastic bag. A plastic tube containing 30 mL of lactic acid and 0.15 g of 98 atom% Ba¹³CO₃ (Icon Inc., USA) was put into the plastic bag in order to inject the released ¹³CO₂ from the mixed solution of lactic acid and Ba¹³CO₃. Injections of ¹³CO₂ were made in the early morning (6-7 o'clock) for approximately one hour so that the *E. japonicum* could uptake sufficient ¹³CO₂ on two successive sunny days as previously described (Ida and Kudo 2008). ¹³C labelled plants were harvested around sunset (17-18 o'clock) on the second day of labelling. Twelve additional unlabelled flowering plants were collected in order to measure baseline ¹³C levels, which was the ratio of atmospheric ¹³CO₂ to ¹²CO₂. The harvested plants were separated into flower or fruit, leaf, aerial stem, underground stem, and bulb, and oven-dried at 70°C for 72 hours, followed by grinding in order to measure the $\delta^{13}\text{C}$ (‰) using an isotope ratio mass spectrometer (IsoPrime-EA, Micromass Ltd., Manchester, UK).

The carbon allocation ratio to each organ was estimated using the $\delta^{13}\text{C}$. First, R_{sample} was calculated from the $\delta^{13}\text{C}$ and it was used to calculate the atom% of ¹³C as follows (Ida and Kudo 2008):

$$R_{\text{sample}} = \frac{{}^{13}\text{C}}{{}^{12}\text{C}} = \frac{\delta^{13}\text{C}(\text{‰}) + 1000}{1000} \times R_{\text{standard}} \quad (1)$$

where R_{sample} is the ratio of ¹³C and ¹²C in each organ, and $R_{\text{standard}} = 0.0112372$, which is the carbon isotope ratio of the international VPDB standard. The ¹³C abundance in each organ was indicated as atom% of ¹³C and calculated from R_{sample} as follows (Hasegawa et al. 2003):

$$\begin{aligned} \text{atom\% of } {}^{13}\text{C} &= \frac{\text{amount of } {}^{13}\text{C}}{\text{amount of } {}^{12}\text{C} + \text{amount of } {}^{13}\text{C}} \times 100 \\ &= \frac{R_{\text{sample}}}{R_{\text{sample}} + 1} \times 100 \end{aligned} \quad (2)$$

The increment of ¹³C content ($C_{\text{increment}}$) in each organ of the

leveled plants was calculated from the atom% of ¹³C as follows:

$$C_{\text{increment}} = \frac{A_{\text{label}} - A_{\text{control}}}{100} \times W \times C_{\text{percentage}} \quad (3)$$

where A_{label} is the atom% of ¹³C in each organ of the labelled individuals, A_{control} is the atom% of ¹³C in each organ of the unlabelled individuals, W is the biomass of each organ (mg), and $C_{\text{percentage}}$ is the carbon concentration of each organ. The ratio of the increment of ¹³C content in each organ (organ $C_{\text{increment}}$) to that of entire plant (plant $C_{\text{increment}}$), ¹³C (‰), was calculated as follows:

$${}^{13}\text{C}(\text{‰}) = \frac{\text{organ } C_{\text{increment}}}{\text{plant } C_{\text{increment}}} \times 100 \quad (4)$$

Seed Production and Seed Test

The fruits and seeds of *E. japonicum* were collected in mid-June when seed dispersal occurred from both the ambient and shade treatment plots in order to understand the effects of early shading on seed production and seed quality.

The collected seeds were moved to the laboratory, and the numbers, size, and weight of the seeds were measured (ISTA 2010). The seed length and seed width were measured using Vernier calipers. The fruit size was estimated by the formula for the volume of an ellipsoid, assuming a prolate spheroid, as follows;

$$V_f = \frac{4}{3} \pi a b^2 \quad (5)$$

where V_f is the fruit volume, a is the length of the major axis, and b is the length of the minor axis. The length of the axes was measured using Vernier calipers. The percent fruit set was calculated as the percent of individuals with fruits out of the individuals with flowers in each subplot, because each *E. japonicum* produces only one flower per year. The mature and immature seeds in an ovule were counted and the mature seed set was calculated (Burd 1994). Ten seeds per each individual were randomly selected and dried at 70°C for 72 hours and the dry weight was measured. A tetrazolium test was conducted to test the seed viability at 30°C for 24 hours using 1% 2, 3, 5-triphenyl tetrazolium.

Data Analysis

The soil environments and LAI between the ambient and shade treatments in each study site were compared using an independent *t*-test.

An independent *t*-test was conducted in order to compare the aerial and underground stem length, specific leaf area, individual biomass, fruit set, fruit mature rate, fruit size, seed number per fruit, seed size, percent mature seed set, biomass of each organ, and ¹³C ratio of each organ between the ambient and shade treatments in early-May, mid-May, and late-May. The aerial stem length of the flowering plants in late-May and the aerial stem biomass of the non-flowering plants in mid-May and late-May were analyzed using the Mann Whitney *U*-test to compare ambient and shade treatment results, because the data did not follow normal distribution. The wilting and dead leaves between the ambient and shade treatments were compared using the χ^2 test. One-way ANOVA was conducted in order to compare the periodic changes in the biomass of each organ among early-May, mid-May, and late-May within the same treatment. The Tukey test was used for post hoc comparisons. The Kruskal-Wallis test was conducted for the comparison of periodic differences in the aerial stem biomass of the non-flowering plants in both the ambient and shade treatments. Orthogonal contrast analysis was used to compare the leaf senescence between the ambient and shade treatments. The

significance was set at $p < 0.05$. The descriptive statistics were conducted using Microsoft Excel 2007 (Microsoft Inc. 2007), and the program SPSS 21.0 was used for the ANOVA and post hoc tests (IBM SPSS Statistics for Windows, Version 21.0. IBM Corp., Armonk, USA).

Conclusions

Earlier leaf flushing and canopy closure could reduce radiation to the spring ephemeral *E. japonicum*. The leaf area and specific leaf area grew larger, such as that of shade leaves, under the shade treatment. However, leaf senescence and death came earlier, contributing to lower current year photosynthetic production, which resulted in smaller fruit and seed biomass under the shade treatment. *E. japonicum* used photosynthates stored during the previous year for aboveground vegetative growth and current-year photosynthates for reproduction. Therefore, current-year photosynthetic production is important for the reproduction of *E. japonicum*.

Although plants under early shading put priority on current-year photosynthate allocation to reproduction, the low photosynthetic production reduced seed production in quantity and quality, which could result in a decrease in the germination rate and population size during the next year. The results of this study implied a possible difficulty in the maintenance of the spring ephemeral *E. japonicum* population under unstable changes in the light conditions due to climate change, such as the earlier arrival of spring and shortened leaf flushing period. The results of this study suggest the importance of conservation measures for spring ephemerals sensitive to climate change.

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Author's Contributions

HJK and PSP conceived and designed the experiments. HJK carried out field work, data analysis and wrote the manuscript. JBJ and YLJ contributed to field work and data analysis. JHS contributed to data analysis and discussion. PSP supervised the study, did additional data analysis and reviewed and edited the work.

References

Augsburger CK (1984) Light requirements of neotropical tree seedlings: a comparative study of growth and survival. *J Ecol* 72:777–795

- Beaubien E, Freeland H (2000) Spring phenology trends in Alberta, Canada: links to ocean temperature. *Int J Biometeorol* 44:53–59
- Benvenuti S, Macchia M, Stefani A (1994) Effects of shade on reproduction and some morphological characteristics of *Abutilon theophrasti* Medicos, *Datura stramonium* L. and *Sorghum halepense* L. Pers. *Weed Res* 34:283–288.
- Burd M (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot Rev* 60:83–139
- Blodgett FH (1910) The origin and development of bulbs in the genus *Erythronium*. *Botanical gazette* 50:340–373
- Dale MP, Causton DR (1992) The ecophysiology of *Veronica Chamaedrys*, *V. Montana* and *V. Officinalis*. III. effects of shading on the phenology of biomass allocations – a field experiment. *J Ecol* 80:505–515
- Easu K (1965) *Plant Anatomy*. John Wiley & Sons, Inc., New York
- Eickmeier WG, Schussler EE (1993) Responses of the spring ephemeral *Claytonia virginica* L. to light and nutrient manipulations and implications for the “vernal-dam” hypothesis. *Bull Torrey Bot Club* 120:157–165
- Fitter AH, Fitter RSR (2002) Rapid changes in flowering time in British plants. *Science* 296:1689–1691
- Gandin A, Dizengremel P, Lapointe L (2011) Photoperiod has a stronger impact than irradiance on the source–sink relationships in the sink-limited species *Erythronium americanum*. *Botany* 89:763–770
- García MB, Ehrlén J (2002) Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. *Am J Bot* 89:1295–1302
- Ghering JL, Delph LF (2006) Effects of reduced source-sink ratio on the cost of reproduction in females of *Silene latifolia*. *J Plant Sci* 167:843–851
- Hasegawa S, Koba K, Tayasu I, Takeda H, Haga H (2003) Carbon autonomy of reproductive shoots of Siberian alder (*Alnus hirsuta* var. *sibirica*). *J Plant Res* 116:18–188
- Horibata S, Hasegawa SF, Kudo G (2007) Cost of reproduction in a spring ephemeral species, *Adonis ramosa* (Ranunculaceae): carbon budget for seed production. *Ann Bot* 100:565–571
- Humbeck K (2013) Epigenetic and small RNA regulation of senescence. *Plant Mol Biol* 82:529–537
- Ida TY, Kudo G (2008) Timing of canopy closure influences carbon translocation and seed production of an understory herb, *Trillium apetalon* (Trilliaceae). *Ann Bot* 101:435–446
- ISTA (2010) *International Rules for Seed Testing*. International Seed Testing Association, Bassersdorf, Switzerland
- Kawano S (2005) Life-history monographs of Japanese plants. 1: *Erythronium japonicum* Decne. (Liliaceae). *Plant Spec Biol* 20:67–74
- Kawano S, Hiratsuka A, Hayashi K (1982) Life history characteristics and survivorship of *Erythronium Japonicum*. The productive and reproductive biology of flowering plants V. *Oikos* 38:129–149
- Kim SY, Lee SY, Rhie YH, Kim KS (2014) Breaking bud dormancy in *Erythronium japonicum* Decne. (Liliaceae) by natural and artificial chilling. *Hort Environ Biotechnol* 55:380–386
- Kitaoka S, Watanabe Y, Koike T (2009) The effects of cleared larch canopy and nitrogen supply on gas exchange and leaf traits in deciduous broad-leaved tree seedlings. *Tree Physiol* 29:1503–1511
- Korea Meteorological Administration (2014) Weather information. Available at: http://www.kma.go.kr/weather/climate/average_30years.jsp (accessed on 8 January 2014)
- Korea National Arboretum (2011) Reports on the conservation and adaptation projects for the forest species sensitive to climate change. Korea National Arboretum, Pocheon
- Lambers H, Chapin III FS, Pons TL (2008) *Plant Physiological Ecology*, 2nd ed. Springer, New York.

- Lapointe L (2001) How phenology influences physiology in deciduous forest spring ephemerals. *Physiol Plantarum* 113:151–157
- Larcher W (2003) *Physiological Plant Ecology: Ecophysiology and Stress Physiology of Function Groups*, 4th ed. Springer-Verlag, Berlin Heidelberg
- Lichtenthaler HK, Buschmann C, Doll M, Fietz H, Bach T, Kozel U, Meier D, Rahmsdorf U (1981) Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynth Res* 2:115–141
- Lubbers AE, Christensen NL (1986) Intraseasonal variation in seed production among flowers and plants of *Thalictrum thalictroides* (Ranunculaceae). *Am J Bot* 73:190–203
- Lubbers AE, Lechowicz MJ (1989) Effects of leaf removal on reproductions vs. belowground storage in *Trillium grandiflorum*. *Ecology* 70:85–96
- McKenna MF, Houle G (2000) Why are annual plants rarely spring ephemerals? *New Phytol* 148:295–302
- McKenna, MF (1999) The effect of The effect of light on the growth and reproduction of *Floerkea proserpinacoides*. *New Phytol* 141:99–108
- Menzel A (2000) Trends in phenological phases in Europe between 1951 and 1996. *Int J Biometeorol* 44:76–81
- Menzel A, Sparks TH, Estrella N et al. (2006) European phenological response to climate change matches the warming pattern. *Glob Change Biol* 12:1969–1976
- Muller RN (1978) The phenology, growth and ecosystem dynamics of *Erythronium americanum* in the northern hardwood forest. *Ecol Monogr* 48:1–20
- National Academy of Agricultural Science, 2011. Korea Soil Classification and Explanation. National Academy of Agricultural Science, Rural Development Administration, Suwon
- Nishikawa Y (2009) Significance of intra-inflorescence variation on flowering time of a spring ephemeral, *Gagea lutea* (Liliaceae), under seasonal fluctuations of pollinator and light availabilities. *Plant Ecol* 202:337–347
- Park YM, Park PS, Sohng JE, Lee SK, Kim M-J (2010) Changes in growth and reproductive strategy of *Disporum smilacinum* in canopy gap and closed canopy areas. *Hort Environ Biotechnol* 51:463–469
- Risser P, Cottam G (1967) Influence of temperature on the dormancy of some spring ephemerals. *Ecology* 48:500–503
- Routhier MC, Lapointe L (2002) Impact of tree leaf phenology on growth rates and reproduction in the spring flowering species *Trillium erectum* (Liliaceae). *Am J Bot* 89:500–505
- Schemske DW, Willson MF, Melampy MN, Miller LJ, Verner L, Schemske KM, Best LB (1978) Flowering ecology of some spring woodland herbs. *Ecology* 59:351–366
- Sparling J (1967) Assimilation rates of some woodland herbs in Ontario. *Bot Gaz* 128:160–168
- Sunmonu N, Kudo G (2014) How do sink and source activities influence the reproduction and vegetative growth of spring ephemeral herbs under different light conditions? *J Plant Res* 127:503–511
- Vézina PE, Grandtner MM (1965) Phenological observations of spring geophytes in Quebec. *Ecology* 46:869–872
- Vitasse Y, Porté AJ, Kremer A, Michalet R, Delzon S (2009) Responses of canopy duration to temperature changes in four temperate tree species, relative contributions of spring and autumn leaf phenology. *Oecologia* 161:187–198
- Yokoi Y (1976) Growth and reproduction in higher plants II. Analytical study of growth and reproduction of *Erythronium japonicum*. *J Plant Res* 89:15–31
- Yoshie F, Fukuda T (1994) Effects of growth temperature and winter duration on leaf phenology of *Erythronium japonicum*, a forest spring geophyte. *Oecologia* 97:366–368