

Effects of growth temperature and winter duration on leaf phenology of a spring ephemeral (*Gagea lutea*) and a summergreen forb (*Maianthemum dilatatum*)

Fumio Yoshie

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Abstract Effects of growth temperature and winter duration on leaf longevity were compared between a spring ephemeral, *Gagea lutea*, and a forest summergreen forb, *Maianthemum dilatatum*. The plants were grown at day/night temperatures of 25/20°C and 15/10°C after a chilling treatment for variable periods at 2°C. The temperature regime of 25/20°C was much higher than the mean air temperatures for both species in their native habitats. Warm temperature of 25/20°C and/or long chilling treatment shortened leaf longevity in *G. lutea*, but not in *M. dilatatum*. The response of *G. lutea* was consistent with that reported for other spring ephemerals. Air temperature increases as the vegetative season progresses. The decrease in leaf longevity in *G. lutea* under warm temperature condition ensures leaf senescence in summer, an unfavorable season for its growth. This also implies that early leaf senescence could occur in years with early summers. Warm spring temperatures have been shown to accelerate the leafing-out of forest trees. The decrease in leaf longevity due to warm temperature helps synchronize the period of leaf senescence roughly with the time of the forest canopy leaf-out. Prolonged winter due to late snowmelt has been shown to shorten the vegetative period for spring ephemerals. The decrease in leaf longevity due to long chilling treatment would correspond with this shortened vegetative period.

Keywords Forest canopy leafing · Leaf longevity · Leaf senescence · Shade-intolerant · Summer dormancy

Introduction

Spring ephemerals in the temperate deciduous forests exhibit summer dormancy. Their epigeous phase is a short vernal period that occurs prior to canopy closure of the forest (Vézina and Grandtner 1965; Caldwell 1969; Kawano et al. 1978, 1982; Mahall and Bormann 1978; Muller 1978). Dense shade due to forest canopy closure and high temperatures in summer are unfavorable for the growth of spring ephemerals, since they are shade-intolerant (Sparling 1967; Taylor and Pearcy 1976; Kawano et al. 1978, 1982) and high temperatures increase the dark-respiration rate (Kawano et al. 1978). It is important for spring ephemerals to adequately regulate the time of leaf senescence in summer. Late leaf senescence of spring ephemerals will result in a loss of photosynthates because of respiration at high temperatures, while early leaf senescence will result in a lost opportunity for photosynthetic production.

Field studies suggest that leaf senescence of spring ephemerals is not directly controlled by the shade of the forest canopy (Lapointe 2001). In a year when spring conditions develop early, yellowing of *Erythronium umbilicatum* leaves start prior to the canopy leaf-out (Caldwell 1969). The percentage of the forest canopy cover at the initiation of leaf senescence in *E. americanum* varies every year, and the time from canopy closure to senescence varies between years (Muller 1978). The time of leaf senescence in *Erythronium japonicum* growing in deciduous *Quercus mongolica* forests was slightly earlier than in evergreen coniferous *Cryptomeria japonica* forests, with low light conditions throughout the year (Sawada et al. 1997). Spring ephemerals occasionally inhabit the margins and gaps of deciduous forests and evergreen forests, and are subjected to various light conditions at the time of leaf

F. Yoshie (✉)
Institute of Natural Sciences, Senshu University,
Higashi-mita, Tama-ku, Kawasaki 214-8580, Japan
e-mail: yoshie@isc.senshu-u.ac.jp

senescence, making it hard to utilize light as an environmental cue for leaf senescence.

Experimental studies have shown that leaf senescence in spring ephemerals occurred under constant high light conditions (Risser and Cottam 1967; Yoshie and Fukuda 1994; Lapointe and Lerat 2006). This shows that leaf senescence is more or less independent of the light conditions. Leaf longevity and time of leaf death of plants are controlled by many internal and external factors, such as photosynthetic rate, leaf-emergence pattern, temperature, light, drought, nutrients, and so on (Kikuzawa 2005). The most striking feature of the leaf death in spring ephemerals is that it is controlled by growth temperature and winter duration. Warm growth temperatures accelerate leaf senescence in *E. japonicum* (Yoshie and Fukuda 1994) and *E. americanum* (Lapointe and Lerat 2006). Longer chilling treatments accelerate leaf senescence in *Erythronium albidum*, *Dicentra cucullaria* (Risser and Cottam 1967) and *E. japonicum* (Yoshie and Fukuda 1994). However, the ecological significance of these responses is still unclear.

One way to evaluate the ecological significance of such responses is to clarify whether they are specific to spring ephemerals. If the responses of spring ephemerals are associated with summer dormancy, such responses will not be found in summergreen plants. In this study, the responses of leaf longevity to growth temperature and the duration of chilling treatment were compared between a spring ephemeral, *Gagea lutea* (L.) Ker-Gawl., and a forest summergreen forb, *Maianthemum dilatatum* (Wood) Nels. et Macbr. The leaves of *M. dilatatum* die in late summer and early autumn and exhibit higher optimum temperature for photosynthesis and are shade tolerant in summer (Koyama and Kawano 1973); these characteristics are similar to those observed in other forest perennial forbs in which the epigeous phase is terminated by early autumn (Sparling 1967; Taylor and Percy 1976; Kawano et al. 1978).

Materials and methods

Gagea lutea is a bulb geophyte distributed throughout Europe and northeastern Asia and inhabits mainly temperate deciduous forests and forest margins in Japan (Satake 1982; Kawano and Nishikawa 2004). A sexually immature plant has a radical leaf, and a mature plant has a leaf and a flower stalk that senesce simultaneously. *M. dilatatum* is distributed in northeastern Asia and North America and inhabits mainly temperate deciduous and sub-boreal evergreen coniferous forests (Kawano et al. 1968). *M. dilatatum* is a clonal plant and is a rhizomatous geophyte. It has a cordate leaf or a flower stalk with two leaves

that sprouts from the rhizomes. The duration of the epigeous phase of these two species is coincident with their leaf longevity.

Corms of *G. lutea* were collected from an area of approximately 15 m² in a sparse secondary deciduous broad-leaved forest (N43°5', E141°20') located at Sapporo, Hokkaido, northern Japan, a cool-temperate region. The vegetation of the herbaceous layer was dominated by perennial species belonging to Poaceae. The shoots of *G. lutea* were shaded by the leaves of trees and grasses late in the growing season. The corms were collected on 17 October 1993. The ground of the collection site is covered with snow from mid-December to early April. Aerial shoots sprout mainly in mid-April, just after snowmelt, and senesce by mid-June. Leaf longevity is approximately 2 months long. The collected corms were classified into 22 size classes based on their fresh weight, from 1.444 to 1.186 gFW in the largest class (class 1) to 0.080–0.055 gFW in the smallest class (class 22). The corms were divided into ten groups for the experimental treatments (five chilling treatments followed by two temperature treatments). Each group consisted of 22 corms from each size class. These corms were divided such that the difference in the total corm weight among the groups was not significant (mean ± SD = 0.501 ± 0.002 gFW). Totally 22 corms for each treatment were planted in uniform commercial soils that are used for ornamental herbs in three 20-cm-diameter plastic pots (size classes 1–6, 7–13, and 14–22 in each pot). The corms were potted at a depth of 4 cm (from the corm top).

The rhizomes of *M. dilatatum* plants were collected on 16 October 1993 from a site (ca. 4 m × 3 m) in a deciduous broad-leaved forest located in the Tomakomai Experimental Forests of Hokkaido University (N42°42', E141°34'), in the Tomakomai, Hokkaido, cool-temperate region. The ground of the collection site is covered with snow from mid-December to early April. The shoots sprout in late April to early May and senesce by early September. Leaf longevity is approximately 4 months long. Resting buds of *M. dilatatum* are formed on long slender rhizomes that are extensively creeping and branched. In the collection site, a dense and uniform *M. dilatatum* plant community was established, and 20 soil mats (each of approximately 30 cm square and 10 cm deep) including dense intersected rhizomes were collected. Each soil mat was cut to match the pot size and was planted in a 25-cm-diameter plastic pot with the same soils as that used in the pots of *G. lutea*. Totally 20 pots were prepared for 10 treatments (5 chilling treatments followed by 2 temperature treatments), and 2 pots were used for each treatment. The method of harvesting might limit the number of clones per pot, and certain genotypes might influence the treatment response.

The *G. lutea* corms and *M. dilatatum* rhizomes were incubated at 8°C in the pots before the start of the chilling treatment to prevent rapid changes in the temperature. The plants of both species required chilling temperatures for normal sprouting and growth at late October (Yoshie and Yoshida 1989; Yoshie 2008). The chilling treatment of both species started on 4 November. The potted corms and rhizomes were subjected to a chilling temperature of 2°C in the dark in a growth incubator (ICB-301L, Asahi Techno Glass, Tokyo) for five different durations (0, 35, 70, 105, and 140 days). After the chilling treatment, potted plants of both species were cultivated at 15/10°C (day/night) and 25/20°C under 12-h day length in the growth incubator. The fluorescent and incandescent lamps used in the experiment produced photosynthetically active radiation of 200–230 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the pot surface level. The air humidity in the incubator was not controlled. Since air humidity decreases with increasing of temperature, air humidity might affect the response of temperature treatment. No nutrients were supplied throughout the experiment for both species. Average mean and average maximum/minimum air temperatures (from 1971 to 2000) during the main growing months in their native habitats were 11.7°C and 16.4/7.6°C for *G. lutea* (6.7°C and 11.1/2.7°C in April, 12.1°C and 17.0/7.8°C in May, and 16.3°C and 21.1/12.4°C in June) and 15.2°C and 18.3/12.6°C for *M. dilatatum* (9.4°C and 13.2/6.0°C in May, 13.4°C and 16.4/10.9°C in June, 17.7°C and 20.4/15.7°C in July, and 20.3°C and 23.0/17.8°C in August). Temperatures of 25/20°C were considerably higher than the mean natural temperature during vegetative growth of both species.

Leaf phenological traits were observed and recorded in the same manner for both species. After the leaves emerged above the soil, the length of leaves was measured at intervals of 1 to 2 days until they stopped growth, and the rate of dying (10% intervals) was measured at intervals of 1–2 days until all of them had senesced. The width of the leaves and length of the petioles in *M. dilatatum* were also measured at the same intervals until they stopped growth. The number of days required for 5 and 95% extension of the final leaf length and required for 5 and 95% death of total leaf area was judged from the curves of extension and senescence, respectively. The length of the developmental phase was determined to be the period between the time of 5 and 95% extension, the length of the mature phase was expressed as the period between the time of 95% extension and 5% death, and the length of the senescent phase was expressed as the period between the time of 5 and 95% death. Leaf longevity was the sum of the length of these three phases. The mean rate of extension during the developmental phase and the mean rate of senescence during the senescent phase were calculated.

The differences in phenological traits among the treatments were analyzed using two-way ANOVA followed by LSD tests. For the analysis, the average of phenological traits in each pot was calculated for a total of three replicates per treatment in *G. lutea* and of two replicates per treatment in *M. dilatatum*. For *M. dilatatum*, no plants sprouted at 25/20°C in the absence of chilling treatment, and the treatments in which chilling was not performed were excluded from the analysis.

The flowering of *G. lutea*, which was also observed during the experimental period, was size-dependent. For example, in corms that were subjected to the chilling treatment (for 105 and 140 days), all those over 0.372 gFW (class 13) flowered, whereas all those below 0.1904 gFW (class 18) did not. Since the leaf phenological traits of radical leaves in *G. lutea* were not significantly different between flowering and non-flowering plants with the exception of the percent of sprouting, the data sets of both plants were used for the analysis. In *M. dilatatum*, flowering was rarely observed in all treatment conditions, and the data of non-flowering plants (sprout plus one leaf) were used for the analysis.

Results

Gagea lutea

Two-way ANOVA demonstrated that the days required for sprouting, leaf longevity, the length of growth phase, final leaf length, and growth rate were all affected by the growth temperature, chilling duration, and their interaction (Table 1). The length of the mature and senescent phases was both affected by the growth temperature and chilling duration. The percent of sprouting and dying rate was affected by the growth temperature.

Almost all of the plants sprouted at 15/10°C, irrespective of the chilling duration (Fig. 1a). The percent sprouting of plants at 25/20°C decreased with a decrease in the chilling duration, although the difference was not significant. This was primarily caused by the decrease in the sprouting of small bulbs. For example, in the absence of chilling treatment all the bulbs in the size classes ranging from 11 to 22 did not sprout. The number of days required for sprouting at each growth temperature decreased as the chilling duration increased (Fig. 1b). These results indicate that the plants require chilling exposure for the normal sprouting and growth. The percentage of flowering plants increased pronouncedly at 25/20°C, and the time to flower at each temperature decreased as the chilling duration increased (data are not shown).

Leaf longevity decreased linearly with increasing chilling treatment (Fig. 1c) with the exception of plants that

Table 1 Results of two-way ANOVA for the effect of growth temperature and length of chilling treatment on leaf phenological traits of *Gagea lutea*

	Source	df	Mean-square	F	P
Percent of sprouting	Growth temperature	1	3164.19	7.81	0.011
	Chilling duration	4	856.84	2.11	0.117
	Interaction	4	628.58	1.55	0.226
	Error	20	405.36		
Length required for 5% extension (days)	Growth temperature	1	511.15	9.42	0.006
	Chilling duration	4	11623.00	214.10	<0.001
	Interaction	4	817.79	15.06	<0.001
	Error	19	54.29		
Longevity (days)	Growth temperature	1	9528.24	663.92	<0.001
	Chilling duration	4	244.58	17.04	<0.001
	Interaction	4	130.03	9.06	<0.001
	Error	19	14.35		
Length of growth phase (days)	Growth temperature	1	3236.59	355.50	<0.001
	Chilling duration	4	180.59	19.84	<0.001
	Interaction	4	75.63	8.31	<0.001
	Error	19	9.10		
Length of mature phase (days)	Growth temperature	1	723.79	93.32	<0.001
	Chilling duration	4	36.20	4.67	0.009
	Interaction	4	17.00	2.19	0.109
	Error	19	7.76		
Length of senescent phase (days)	Growth temperature	1	191.39	21.73	<0.001
	Chilling duration	4	26.33	2.99	0.045
	Interaction	4	3.03	0.34	0.845
	Error	19	8.81		
Final leaf length (mm)	Growth temperature	1	46983.93	215.04	<0.001
	Chilling duration	4	3764.95	17.23	<0.001
	Interaction	4	5731.37	26.23	<0.001
	Error	19	218.49		
Mean rate of growth (mm/day)	Growth temperature	1	7.80	10.71	0.004
	Chilling duration	4	23.18	31.84	<0.001
	Interaction	4	9.98	13.72	<0.001
	Error	19	0.73		
Mean rate of dying (% leaf area/day)	Growth temperature	1	208.78	55.76	<0.001
	Chilling duration	4	5.77	1.54	0.230
	Interaction	4	3.53	0.94	0.460
	Error	19	3.74		

did not receive chilling and were cultivated at 25/20°C ($y = -0.180x + 85.080$, $r = 0.994$, $P = 0.001$ at 15/10°C; $y = -0.123x + 48.150$, $r = 0.985$, $P = 0.015$ at 25/20°C). Leaf longevity in plants that received long chilling treatments and were grown at 15/10°C was about 2 months, which was equivalent to that of their native habitat. The decrease in leaf longevity with an increase in chilling duration was largely due to the shortened growth period (Fig. 1d–f). The decrease in growth period explained 80 and 90% of the reduction in leaf longevity at 15/10°C and 25/20°C, respectively. The final leaf length of plants at 15/10°C was not affected by the length

of chilling treatment, whereas the final leaf length at 25/20°C was affected by chilling duration, i.e., it increased with increasing chilling duration (Fig. 1g). The growth rate increased with increasing chilling duration, and the increase was more pronounced at 25/20°C (Fig. 1h). Shorter growth period was correlated with faster growth rate at 15/10°C, and shorter growth period was correlated with faster growth rate and longer leaf length at 25/20°C (Fig. 1d, g, h).

Leaf longevity was shortened markedly at 25/20°C irrespective of the chilling duration (Fig. 1c). The decrease in longevity was attributed to the decrease in growth,

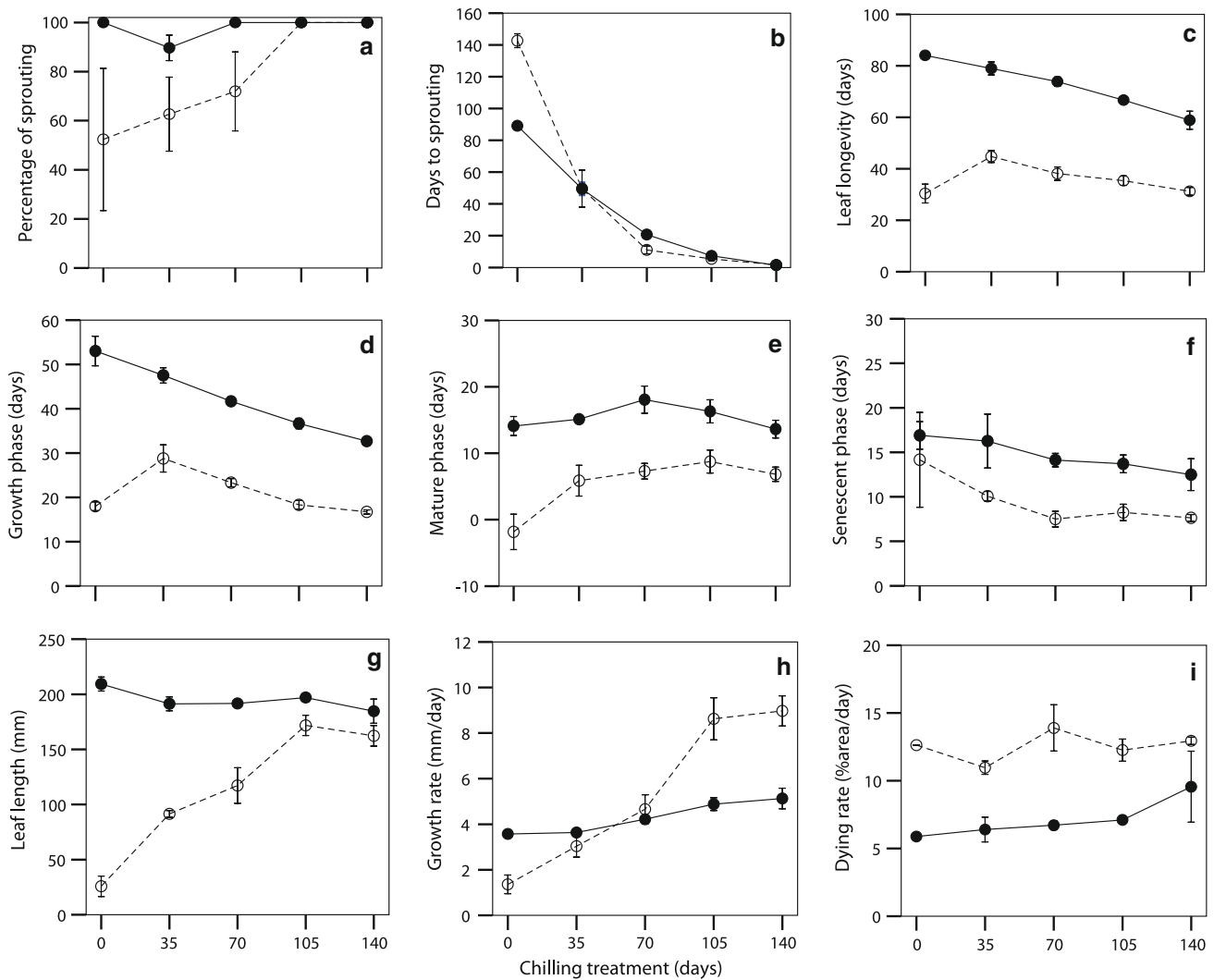


Fig. 1 Responses of leaf phenological traits to growth temperature and chilling duration in *Gagea lutea*. The data shown are mean (\pm standard error) at 15/10°C (closed circles) and at 25/20°C (open circles)

mature, and senescent periods (Fig. 1d–f). Shorter growth period was correlated with slower growth rate and shorter final leaf length in plants submitted to a short chilling period, whereas shorter growth period was correlated with faster growth rate in plants submitted to a long chilling period (Fig. 1d, g, h). The shorter senescent period at 25/20°C compared to that at 15/10°C was attributable to a higher dying rate (Fig. 1f, i).

Maianthemum dilatatum

Days required for sprouting were affected by growth temperature, length of chilling treatment, and their interaction (Table 2). The number of sprouting, length of the growth phase, and growth rate were affected by growth temperature and length of chilling treatment. In addition, leaf longevity, length of the mature phase, and final leaf

width were affected by growing temperature. The length of the senescent phase and dying rate was affected by the length of chilling treatment. The final length of leaves and petioles were not affected by these factors.

In the absence of chilling treatment, no plants sprouted at 25/20°C, and few sprouted at 15/10°C, indicating no or insufficient break of winter dormancy (Fig. 2a). Chilling treatment lasting longer than 35 days markedly increased the number of plants sprouting at both temperatures. Days required for sprouting decreased with increasing chilling duration (Fig. 2b), indicating a progress in breaking the dormancy. Plants at 25/20°C sprouted earlier than those grown at 15/10°C.

Leaf longevity was about 130 days in plants grown at 25/20°C receiving a long chilling treatment (Fig. 2c), which was equivalent to that observed in their native habitat. The mean air temperature during the vegetative

Table 2 Results of two-way ANOVA for the effect of growth temperature and length of chilling treatment on leaf phenological traits of *Maianthemum dilatatum*

	Source	df	Mean-square	F	P
Number of sprouting	Growth temperature	1	61.25	18.28	0.002
	Chilling duration	4	105.95	31.63	<0.001
	Interaction	4	4.00	1.19	0.371
	Error	8	3.35		
Length required for 5% extension (days)	Growth temperature	1	451.24	63.56	<0.001
	Chilling duration	3	370.13	52.14	<0.001
	Interaction	3	45.66	6.43	0.016
	Error	8	7.10		
Longevity (days)	Growth temperature	1	9336.39	559.67	<0.001
	Chilling duration	3	23.66	1.42	0.307
	Interaction	3	38.84	2.33	0.151
	Error	8	16.68		
Length of growth phase (days)	Growth temperature	1	49.07	188.13	<0.001
	Chilling duration	3	1.61	6.16	0.018
	Interaction	3	0.78	3.00	0.095
	Error	8	0.26		
Length of mature phase (days)	Growth temperature	1	9462.43	543.64	<0.001
	Chilling duration	3	19.45	1.12	0.398
	Interaction	3	44.49	2.56	0.128
	Error	8	17.41		
Length of senescent phase (days)	Growth temperature	1	37.82	4.97	0.056
	Chilling duration	3	38.22	5.03	0.030
	Interaction	3	11.89	1.56	0.272
	Error	8	7.60		
Final leaf length (mm)	Growth temperature	1	12.43	1.20	0.305
	Chilling duration	3	40.19	3.89	0.055
	Interaction	3	15.00	1.45	0.300
	Error	8	10.34		
Final leaf width (mm)	Growth temperature	1	41.93	8.77	0.018
	Chilling duration	3	17.21	3.60	0.065
	Interaction	3	4.81	1.01	0.439
	Error	8	4.78		
Final petiole length (mm)	Growth temperature	1	0.81	0.02	0.895
	Chilling duration	3	128.39	2.94	0.099
	Interaction	3	53.37	1.22	0.363
	Error	8	43.68		
Mean rate of growth (mm/day)	Growth temperature	1	3.37	65.73	<0.001
	Chilling duration	3	0.30	5.93	0.020
	Interaction	3	0.15	2.89	0.102
	Error	8	0.05		
Mean rate of dying (% leaf area/day)	Growth temperature	1	3.93	2.34	0.165
	Chilling duration	3	12.37	7.35	0.011
	Interaction	3	3.02	1.80	0.226
	Error	8	1.68		

period in the native habitat was much lower than 25/20°C, which indicates that leaf longevity is not affected by warm temperature. Leaf longevity was decreased markedly at 15/

10°C, which was largely attributable to the decrease in the mature period (Fig. 2d–f), indicating an early induction of the senescent phase. The leaf growth period of plants

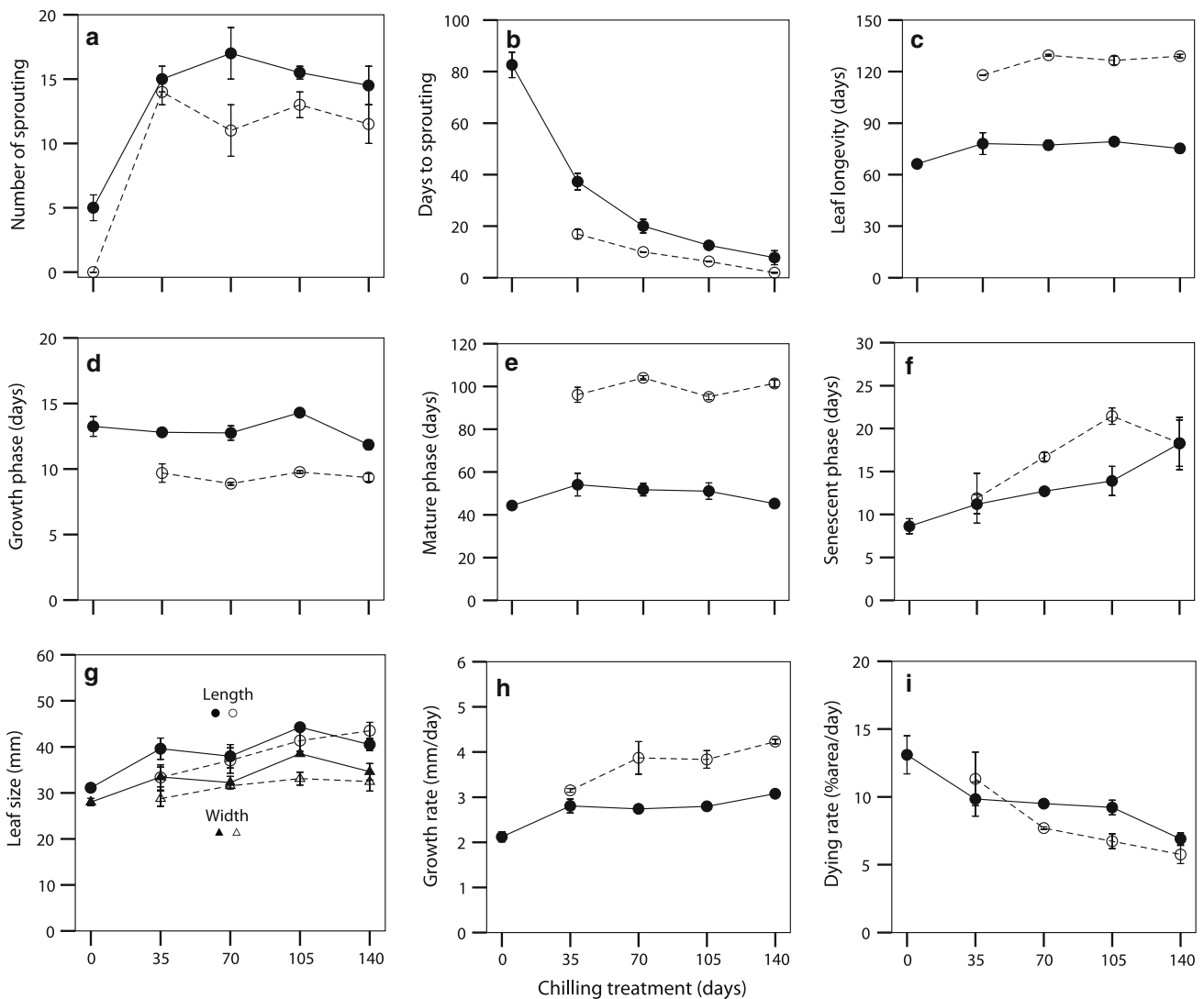


Fig. 2 Responses of leaf phenological traits to growth temperature and chilling duration in *Maianthemum dilatatum*. The data shown are mean (\pm standard error) at 15/10°C (closed symbols) and at 25/20°C (open symbols)

grown at 25/20°C was slightly shorter than that at 15/10°C (Fig. 2d). Shorter growth period was correlated with faster growth rate with no significant impact on leaf length (Fig. 2d, g, h).

Leaf longevity was not affected by chilling duration. The length of growth period did not consistently change with a change in chilling duration (Fig. 2d). The growth rate was affected by chilling duration and increased slightly with an increase in chilling duration (Fig. 2h). The final leaf length and leaf width increased slightly with an increase in chilling duration (Fig. 2g), although the difference was not significant (Table 2). The final petiole length showed responses similar to that observed for the leaf length (data are not shown). The length of the senescent period and the dying rate were affected by the chilling duration. The increase in the duration of the senescent

period with increasing chilling duration was because of the decrease in the dying rate (Fig. 2f, i).

Discussion

This study revealed that warm temperature and long chilling duration shortened leaf longevity in *G. lutea*, but not in *M. dilatatum*. The responses of *G. lutea* were consistent with those of other bulbous spring ephemerals (Risser and Cottam 1967; Yoshie and Fukuda 1994; Lapointe and Lerat 2006), suggesting that they represent traits associated with summer dormancy. Air temperature increases as the vegetative season progresses, which should accelerate the process of leaf senescence. This ensures that leaf senesces in early summer, when conditions become

unfavorable for the growth of spring ephemerals (Sparling 1967; Taylor and Pearcy 1976). The decrease in leaf longevity due to warm temperature also ensures early leaf senescence in years with early summer. This enables spring ephemerals to avoid the loss of photosynthates due to high dark-respiration rate at high temperatures (Kawano et al. 1978). Conversely, the increase in leaf longevity due to cool temperature ensures late leaf senescence in years with late summer. This enables spring ephemerals to utilize a prolonged vegetative period.

The change in leaf longevity due to temperature in spring ephemerals is in contrast with that in *M. dilatatum* and temperate deciduous trees. The leaf longevity in *M. dilatatum* was shortened markedly at 15/10°C. The leaf longevity in *Fagus crenata* seedlings decreased as growth temperature decreased (Yoshie 2006). *M. dilatatum* plants are rarely subjected to temperatures below 10°C in the months of July and August, and a temperature of 15/10°C is probably too cold for normal growth in a late growing season. A strong reduction in leaf longevity at cool temperatures above 10°C was also found in *F. crenata* seedlings cultivated at 11°C (Yoshie 2006). Leaf longevity of plants grown at 25/20°C receiving a long chilling treatment was equivalent to that observed in their native habitat. This indicates that leaf senescence in the native habitat is not caused by a cumulative effect of shade and is more or less independent of the light conditions.

The vegetative period of spring ephemerals lasts for about 2 months before the forest canopy trees leaf out. The leaf-out period of temperate trees can be estimated by summing the air temperature during periods before the leaf-out and by the duration of the winter chilling temperatures (Raulier and Bernier 2000). Warm spring temperatures accelerate the leafing-out process (Murray et al. 1989; Hunter and Lechowicz 1992). Early leafing out of forest trees in a warm spring shortens the bright light period available for the vegetative growth of spring ephemerals (Lapointe 2001). The decrease in leaf longevity in spring ephemerals grown in a warm temperature is in accord with this shortened vegetative period. This response helps synchronize the period of leaf senescence roughly with the time of the forest canopy leafing-out, which is advantageous for the survival of spring ephemerals in the forest floor. The decrease in leaf longevity of *G. lutea* in this study was 2.9 days and that of *E. japonicum* in the previous study was 2.8 days (Yoshie and Fukuda 1994) per 1°C increment of temperature, both of which had received long chilling treatments. During the 30 years between 1971 and 2000, mean air temperatures during the main growing months of *G. lutea* ranged from 10.4°C to 13.5°C (recorded at the Sapporo Meteorological Station located nearest to the native habitat). This difference in temperature potentially results in a 9-day difference in leaf longevity.

The length of winter for spring ephemerals varies every year depending on the temperatures in early spring and early winter. It also varies depending on the timing of snowmelt in early spring for *G. lutea* and other spring ephemerals (Vézina and Grandtner 1965; Muller 1978; Hamerlynck and Smith 1994). Prolonged winter due to late snowmelt could shorten the vegetative period for spring ephemerals. The vegetative period of *E. americanum*, growing in a northern hardwood forest, was shortened by nearly 2 weeks in the year due to 1 month delay in snow cover disappearance. This resulted from about a 2-week delay in the leaf development initiation in *E. americanum*, with no delay in the leaf development process of the canopy trees (Muller 1978). The result indicates that the process of canopy leaf development is more affected by spring air temperatures than the time of snow cover disappearance, and the sprouting of spring ephemerals is delayed by late snow cover disappearance.

The decrease in leaf longevity of spring ephemerals due to long chilling treatment found in this study and the previous studies (Risser and Cottam 1967; Yoshie and Fukuda 1994) would fit with shortened vegetative period due to late snow-cover disappearance, which is also advantageous for the survival of spring ephemerals. Conversely, early snow-cover disappearance lengthens the vegetative period of spring ephemerals. In addition, a short winter may lengthen the vegetative period for spring ephemerals because of late leafing-out of forest trees, since some temperate deciduous trees delay leafing with decreased winter chilling (Cannell and Smith 1986; Murray et al. 1989). The decrease in leaf longevity of *G. lutea* cultivated at 15/10°C was 0.18 day in this study and that of *E. japonicum* cultivated at 15°C was 0.45 day (Yoshie and Fukuda 1994) per 1-day increment of chilling treatment. During the 30 years between 1971 and 2000, the day when snow cover disappeared ranged from 13 March to 17 April at the Sapporo Meteorological Station. This difference in day of snow-cover disappearance potentially results in a 6.3-day difference in leaf longevity of *G. lutea*.

The responses of spring ephemerals observed under controlled growth conditions are consistent with leaf phenological patterns reported in natural habitats. The mature phase of leaves in *E. americanum* was shortened in a year when sprouting was delayed by a late snowmelt (Muller 1978), which might have resulted from being subjected to long chilling temperatures under snow cover. The leaf longevity in *E. japonicum* was shortened when they sprouted late (Fukuda 1987). The decrease in leaf longevity in plants that sprouted later might also be due to warmer air temperature conditions. The responses of spring ephemerals in this study indicate that shorter winters and subsequent cooler vegetative seasons increase leaf longevity. Indeed, such environments in natural habitats

maximize the length of the growing season in spring ephemerals and lead to a high storage of reserves in the bulbs (Rees 1972; Muller 1978; Nault and Gagnon 1988, 1993). In *E. americanum*, the bulb biomass increased on cultivation in cooler temperatures (Lapointe and Lerat 2006).

The recent global warming trend is considered to have caused an accelerated spring leafing and a delayed leaf senescence of temperate deciduous trees (Menzel 2000; Peñuelas et al. 2002). Global warming decreases winter duration and advances the start of spring and summer. These conditions will also advance the vegetative period for *G. lutea* and *M. dilatatum*. Advancement of the vegetative period will endanger the plants, since a decrease in winter duration could impede normal sprouting, normal leaf growth, and normal flowering.

Some bulbous spring plants that exhibit summer dormancy and originate in Mediterranean climatic regions showed responses similar to those of forest spring ephemerals. Warm air temperatures shorten the leaf longevity in *Crocus vernus* (Badri et al. 2007) and tulips (Rees 1972). The plastic responses of summer dormancy in these plants ensure their ability to avoid hot and dry summers. A long chilling duration shortens the growth period and/or increases the growth rate of the leaves of two *Allium* species (Zimmer et al. 1985; De Hertogh and Zimmer 1993). The results may suggest that the responses found in spring ephemerals are common to spring bulbous plants with summer dormancy. Rhizomatous spring ephemerals, such as species of *Anemone* and many summergreen perennial herbs, exist in temperate deciduous forests. Further study on the responses in these species is needed to clarify whether the responses are characteristic to spring ephemerals.

Warm temperature increased the growth rate and shortened the growth period in the leaves of *G. lutea* in this study and *E. japonicum* (Yoshie and Fukuda 1994). These responses were also found in *M. dilatatum* in this study and in summer annuals (Dennett and Auld 1980; Rawson and Hindmarsh 1982), indicating that they are not specific to spring ephemerals. The decrease in the mature and senescent periods in the leaves of *G. lutea* in this study and *E. japonicum* (Yoshie and Fukuda 1994) at warm temperatures is caused by an activation of metabolic processes of the rate of aging, resulting from either a direct effect of temperature or from an indirect effect through a reduction in the sink demand as suggested by Lapointe (2001). At higher cultivation temperature corm growth of *Crocus vernus* stopped before the first visual sign of leaf senescence, suggesting that corm growth controls leaf longevity rather than the opposite and that growth becomes rapidly sink limited (Badri et al. 2007).

Spring ephemerals grow very slowly below the ground surface, but require chilling temperatures for normal

sprouting and growth (Fig. 1a, b). Winter chilling temperatures activate the metabolic processes for the early growth phase of temperate plants through an increase in gibberellic acid and a decrease in abscisic acids, as is well known. The findings of the present and of the previous studies (Risser and Cottam 1967; Yoshie and Fukuda 1994) demonstrate that winter chilling temperatures also activate metabolic processes affecting the rate of aging throughout the life span of leaves in spring ephemerals. The physiological mechanism is still unknown, although it will be different from the activation mechanism by warm temperatures and that for growth initiation.

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