Late canopy closure delays senescence and promotes growth of the spring ephemeral wild leek (*Allium tricoccum*).

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Abstract

Spring ephemerals take advantage of the high light conditions in spring to accumulate carbon reserves through photosynthesis before tree leaves unfold. Recent work reports delayed leaf senescence under constant light availability in some spring ephemerals, such as wild leek (*Allium tricoccum*). This paper aims at establishing if tree canopy composition and phenology can influence the growth of spring ephemerals through changes in their phenology. Wild leek bulbs were planted in 31 plots in southern Québec, Canada, under canopies varying in composition and densities. Light availability and tree phenology were measured along with other environmental conditions and their effect on wild leek growth was assessed with a redundancy analysis. Higher light availability resulted in a better growth of wild leek. The plants postponed their senescence under late bud-bursting trees. Better bulb growth and seed production were then achieved. Tree litter and soil temperature and moisture levels also influenced wild leek growth and survival. Tree leaf phenology thus appears to have a strong impact on spring ephemeral growth by modulating the length of their growing season and their photosynthetic capacity. This underlines the importance of considering the variation of light availability throughout the growing season in the study of spring ephemerals.

Keywords

*Allium tricoccum*; Tree canopy; Light; Phenology; Spring ephemeral
Introduction

The light environment in the understory of deciduous forests varies greatly, both temporally and spatially, even during springtime. The presence of canopy gaps increases light availability, which often benefits the understory vegetation (Canham, 1988a). Furthermore, the timing of canopy closure varies with tree species, which can influence understory vegetation growth (Kato and Komiyama, 2002). Indeed, the duration of the period between snowmelt and canopy closure has been shown to influence growth and reproduction of early summer species (as defined by Givnish, 1987), such as red trillium (*Trillium erectum* L.; Routhier and Lapointe, 2002), and influence photosynthetic capacity and assimilated carbon translocation patterns of understory herbs (Ida and Kudo, 2008; Kim et al., 2015).

Early leafing, as has been observed in spring ephemerals, is a common shade-avoidance strategy for the understory vegetation (Lopez et al., 2008). These plants take advantage of the high light conditions between snowmelt and canopy closure to accumulate photosynthates (Lapointe, 2001) and exhibit higher photosynthetic capacity than summer-green and semi-evergreen species that are found in similar habitats (Rothstein and Zak, 2001a). Nevertheless, they can still be influenced by light availability during springtime. In a recent experiment under controlled light conditions, we demonstrated the positive effect of increased light availability on the growth of the wild leek *Allium tricoccum* Ait. (Amaryllidaceae; Dion et al., 2016). However, experiments in natural environments involving spring ephemerals, such as wild leek, did not report a clear relationship between their growth rate and light availability prior to canopy closure (Vasseur and Gagnon, 1994; Delagrange et al., 2013). Furthermore, they did not take into account variation in tree leaf phenology. Under constant light and temperature, wild leek senescence is delayed relative to its phenology under natural conditions (Bernatchez and Lapointe, 2012). This
delay suggests plasticity in its phenology. In turn, phenology could possibly acclimate to late
canopy closure and provide a longer active period of photosynthesis and, therefore, greater
underground storage at the end of the growing season. Thus, duration of the high light period
might be as important as the instantaneous amount of light that is received in spring for the
herbaceous layer.

Forest canopy composition also influences other environmental variables such as litter
abundance, which in return can influence temperature and moisture in the uppermost soil layers
(Facelli and Pickett, 1991; Barbier et al., 2008). A sparse canopy can modulate the abundance of
summer-green species that compete with spring ephemerals for light and nutrients (Plue et al.,
2013). Tree composition can both affect and be influenced by soil nutrient and water availability
(Barbier et al., 2008; Lukac and Godbold, 2011; Thomaes et al., 2013). Therefore, different
canopies may reflect not only different light environments, but also different soil types that could
also affect the growth of herbaceous understory species.

The general objective of this research was to determine the influence of canopy
composition, which modulates tree phenology and light availability, on spring ephemeral growth.
An experiment with transplanted wild leek bulbs was established to test the combined effects of
light availability and tree leaf phenology in sites that exhibited high light variability amongst one
another, but moderate environmental variability in terms of nutrient availability, soil pH and soil
moisture. Consequently, a multivariate approach was used to take into account differences in the
percentage cover of litter and soil temperature, pH and moisture, together with light conditions.
Furthermore, we measured leaf chlorophyll a/b ratios and bulb δ^{13}C to confirm that wild leek
acclimated to the prevailing light conditions in the different plots. We posit that 1) higher light
availability in spring should increase spring ephemeral growth; the perennial organ of plants
growing under a sparse canopy or gaps should thus get bigger. 2) A delay in canopy closure should also favour spring ephemeral growth. 3) Spring ephemerals should postpone senescence under a forest cover that is composed of late bud-bursting species (e.g., Fraxinus spp., Quercus rubra L.).

Material and Methods

Study species

Wild leek, also commonly known as ramp, is a spring ephemeral geophyte that is very popular in the cuisine and folk medicine of the central Appalachian Mountains of North America, as it is the first edible forest herb to emerge in spring (Davis and Greenfield, 2002; Cavender, 2006). Both its bulb and leaves are edible. Typical of spring ephemerals, the leaves of wild leek unfold early in spring and senesce shortly after canopy closure is completed (Lapointe, 2001). In contrast to most other spring ephemerals that flower in early spring, anthesis in wild leek occurs later in the summer and seed production is completed in early autumn (Jones, 1979). Wild leek has previously been used in comparative studies as being representative of the spring ephemeral guild (Rothstein and Zak, 2001a,b).

Study sites

Experiments were conducted in l’Estrie (the Eastern Townships) and Basses-Laurentides (Lower Laurentian) regions of southern Québec, Canada. These provincial administrative regions exhibit similar climatic conditions. Estrie (plots are located between 45°05’ and 45°25’ N, and from 71°20’ to 71°60’ W) has an annual precipitation of 1144 mm and an average annual temperature of 4.1 °C, accumulating 1637 degree-days (above 5 °C) each year. Basses-Laurentides region (45°25’ to 45°40’ N; 74°00’ to 74°10’ W) has 1065 mm of precipitation, an
average annual temperature of 5.0 °C, and 1866 degree-days over 5 °C (Environment Canada, 2013). Both regions are located close to the northern limit of wild leek’s geographic range and encompass several large natural populations of the species (NatureServe, 2015).

Experimental plots

During spring 2011, we surveyed many publicly and privately owned forests to identify plot areas that exhibited divergent canopy openings, canopy gaps, and overstory tree species composition. We also included tree plantations, in order to cover a wider range of light conditions. Within 13 sites, 47 plot-sized areas were pre-selected. Based on pH measurements and Mehlich-3 extractions (analyses performed by Agri Quanta Inc., St-Ours, QC, Canada), those plots with low pH or severe mineral deficiency or toxicity were excluded in favour of those plots where conditions were suitable for wild leek growth. Thirty-one plots were retained and are detailed in Supplementary Table S1. The exact locations of the plots have not been disclosed to prevent unauthorised harvesting on these sites.

With permission of provincial governmental authorities (the species is protected in Quebec; harvesting is allowed for research purposes), wild leek bulbs were harvested from a single site in Estrie on 30 May 2011. All bulbs were Allium tricoccum var. tricoccum (Jones, 1979). Bulbs were first sorted. Medium-size bulbs were identified and were replanted together, along with all the other bulbs that were harvested, in a nearby forest stand to complete their senescence.

Transplantation was conducted during the third week of August 2011. Bulbs were excavated, floral scapes (elongated, leafless flowering stems) were removed when present, and

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2 Supplementary data are available with the article through the journal Web site.
bulbs were maintained in plastic bags in the dark under cool temperatures during transport. On each selected site, a $1 \times 3$ m plot was delimited and tree saplings directly above it were cut. The soil was plowed to a 15-cm depth and fertilizer was applied following the recommendations of Bernatchez et al. (2013). The soil amendments consisted of 3000 kg·ha$^{-1}$ of gypsum (Uncalcined Gypsum Products, CaSO$_4$, Georgia-Pacific Gypsum Corporation, Atlanta, GA, USA) and 550 kg·ha$^{-1}$ of N-P-K fertilizer (5–10–8 from a mixture of Bio-Jardin 4–3–6 [N–P$_2$O$_5$–K$_2$O], which also contained 3 % Mg, and fossil bone 0–13–0, McInnes Natural Fertilizers Inc., Stanstead, QC).

In each plot, 90 bulbs were planted at a depth of 5 cm and spaced 15 cm apart. Four plots contained only 85 bulbs, due to a shortage of bulbs of the appropriate size. Natural litter was set aside prior to plowing and repositioned on top of the plots after planting was completed. Manual weeding was performed during plot establishment. Percentage weed cover was estimated immediately prior to weeding, which took place after wild leek had senesced in 2012 and 2013.

**Plant growth measures**

Growth of wild leek was measured in 2012 and 2013, and included total leaf width (TLW; measured in early May, with a ruler), bulb width (early July, after complete leaf senescence, with calipers and minimum soil disturbance), number of bulbs (early May; estimated from the number of individual living plants), number of flowering scapes per plot (early July), and seed number per plant (end of August). Total leaf width has been previously correlated with plant biomass (Nault and Gagnon, 1993).

Weekly surveys were performed in all plots in 2012 and 2013, during which we estimated the number of plants in each of the following phenological stages: 1 = leaf emergence; 2 = leaf unfolding; 3 = fully expanded leaves; 4 = beginning of senescence, with < 25% of leaf area.
senesced; 5 = advanced senescence, with > 25% leaf area senesced; 6 = fully senesced leaves (Fig. 1). Surveys were converted to percentages of individuals at a given stage. Because our objective was to assess the duration of wild leek photosynthetic activity and to transform these phenology surveys into a single value that was usable in linear correlation and redundancy analysis, we developed a *Wild Leek Phenology Index* (WLPI), as follows:

\[
WLPI = \sum_{i=1}^{n} \left( N_i (S_{3i} + 0.595S_{4i} + 0.255S_{5i}) \right)
\]

where \( n \) is the number of surveys that were performed during the growing season; \( N_i \) is the number of days accounted for by the survey, i.e., from the middle of the period between the \((i-1)^{th}\) and the \(i^{th}\) surveys to the middle of the period between the \(i^{th}\) and the \((i+1)^{th}\) surveys (the first and last periods respectively started or ended on the first and last surveys); \( S_{3i}, S_{4i} \) and \( S_{5i} \) are respectively the percentages of wild leek individuals at phenological stages 3, 4 and 5 during the \(i^{th}\) survey. Spring ephemeral leaves attain their maximum photosynthetic rate at the beginning of stage 3 (Lapointe, 2001). During stages 4 and 5, photosynthetic capacity drops to 59.5 and 25.5% of the maximum capacity that is recorded during stage 3, based upon data that have been reported previously (Bernatchez and Lapointe, 2012); hence, the inclusion of the constants in equation 1. However, it should be stressed that the data from Bernatchez and Lapointe (2012) were taken in growth chambers providing constant light and temperature. The declining light and rising temperature that is usually observed in deciduous forests during spring may affect variation in photosynthetic capacity, thereby modifying the constants used in equation 1. Unfolding leaves usually act as C sinks for spring ephemerals, as has been demonstrated in the yellow trout lily *Erythronium americanum* Ker Gawler (Gandin et al., 2011). Therefore, stages 1 and 2 were not included. The last survey took place immediately following complete leaf senescence.
In order to confirm that plants had acclimated to the local plot light environment, we analysed leaf chlorophyll a/b ratios (chl a/b) and bulb stable carbon isotopic composition (δ^{13}C) in 2013 (Year 2). Chlorophyll a/b ratios are known to increase under high light conditions (Dale and Causton, 1992). We randomly sampled four leaves from different plants in each plot, after canopy closure, and kept them frozen at -15 °C until measurements could be made. Following acetone extraction, chlorophyll absorbance of the supernatant was measured by spectrophotometry and quantified using the equations of Porra and Grimme (1974).

We measured the δ^{13}C in the bulb as an indicator of variation in mean carbon fixation rates among plots throughout the growing season. The value of δ^{13}C usually increases with photosynthetic activity, because RUBISCO discriminates less against ^{13}C relative to ^{12}C as the activity of the enzyme increases (Dawson et al., 2002; Bowling et al., 2008). We preferred to quantify δ^{13}C in the bulb rather than in the leaf, as leaf δ^{13}C mostly reflects intercellular carbon concentration at the time of leaf formation (Dawson et al., 2002). We harvested four bulbs per plot after complete leaf senescence in early July 2013. They were kept refrigerated for less than 4 days, dried at 70 °C for 48 h, and ground to a powder with a mortar and pestle. Samples were encapsulated in tin and sent to the Stable Isotope Facility at the University of California, Davis, for ^{13}C analysis (PDZ Europa ANCA-GLS elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer; Sercon Ltd., Crewe, Cheshire, UK).

Light estimation

Four hemispherical photographs of the canopy were taken above each plot throughout spring 2013 using a Nikon CoolPix 4500 camera and a Nikon FC-E8 hemispherical lens (Nikon Inc., Tokyo, Japan). The lens was positioned 80 cm above the centre of the plot with the bottom
of the picture oriented towards magnetic north using a compass. The first series of photographs were taken before tree leaf unfolding, with the second and third series being taken during tree leaf unfolding, and the fourth series was taken after full canopy closure of all tree species. To prevent direct light from affecting image quality, photographs were taken under a completely overcast sky when possible, or at dawn or dusk. Using a Garmin eTrexH GPS navigator (Garmin Ltd., Olathe, KS, USA), exact position and elevation were determined. The degree and direction of ground slope were measured using a clinometer and compass.

The photographs were analysed using Gap Light Analyzer 2.0 software (Frazer et al., 1999). Since an exact cloudiness index was unavailable for the regions under study, we estimated it using the monthly sun fraction that had been calculated for these regions by Gariépy et al. (1981), together with the Glower and McCulloch model, as described in Besharat et al. (2013). The geographical position, slope degree and direction, date and cloudiness index allowed the software to calculate the sun path and to model direct, diffuse and total solar radiation that was transmitted through canopy to reach the ground.

Total light reaching the plot during wild leek growing season was modelled by multiplying the calculated daily solar radiation by the number of days that each photograph represented. We defined the period between complete snowmelt (19 April) and complete leaf senescence of wild leek (15 June) as the 2013 growing season. Although wild leek phenology could also respond to the ratio of red to far-red light (R/FR; Lavallée, 1978; von Wettberg and Schmitt, 2005), it is well established that R/FR is closely related to total light availability (Capers and Chazdon, 2004; Yirdaw and Luukkanen, 2004).
Canopy phenology status was quantified by estimating the mean leaf width of the main tree species surrounding each plot. We developed a *Canopy Closure Index* (CCI) using the formula:

\[
CCI = \sum_{i=1}^{n} \left( \frac{L_i}{L_{max}} \times N_i \right) ^ 7
\]

where \( n \) is the number of surveys that were performed during the growing season (the last one immediately after complete wild leek leaf senescence); \( L_i \) is the mean tree leaf width at survey \( i \); \( L_{max} \) is the tree leaf width after complete unfolding; and \( N_i \) is the number of days that each survey covered, which was calculated in the same manner as the WLPI. A higher CCI indicates that canopy closure occurs sooner.

**Other abiotic factors**

The percentage of the plot area covered by litter was noted for each plot during weekly surveys. Soil volumetric water content was measured using a FieldScout TDR 300 soil moisture meter (Spectrum Technologies, Inc., Aurora, IL, USA) at each visit. We buried temperature sensors (Thermochron iButton DS1921G, Maxim Integrated Products Inc., San Jose, CA, USA) at ca. 5 cm depth. These sensors were sealed in plastic bags before burial near each plot, immediately after 2013 snowmelt. The sensors recorded hourly temperature throughout wild leek growing season.

**Statistical analyses**

**Redundancy analysis**

Given the number of quantitative variables that were recorded, we performed a redundancy analysis (RDA) on the environmental data (total light, CCI, light availability after...
canopy closure, soil temperature average and variance, soil moisture, litter percent cover, weed percent cover and pH) and wild leek growth responses (total leaf width per plant, WLPI, individual bulb width, number of bulbs per plot, number of seeds per plant). RDA was performed within the **vegan** package (Oksanen et al., 2012) of R (version 2.15.1; R Development Core Team, 2012), following programming directions from Borcard et al. (2011) to determine the main trends. We also tested the main hypothesis and predictions using linear regression. Transformations were performed on the following environmental variables to respect normality assumptions: natural logarithm (ln(x); light availability after canopy closure), inverse transformation (-1/x; total light and soil moisture), omnibus normalizing procedure (rank transformation respecting the normal distribution, see Legendre and Legendre, 2012; litter), and square root (√x; mean daily variance for soil temperature).

**Results**

**Redundancy analysis**

Triplots of the RDA are presented in Fig. 2. Source data are presented as Supplementary Table S1. The RDA ordination was significant (permutation test, $F_{8, 21} = 4.986, P < 0.001$; adjusted $R^2 = 0.524$); 52.4 % of the variation in the response data (i.e., wild leek growth) was explained by variation in the environmental data. Four canonical axes were significant ($P < 0.05$) and respectively explained 31.98, 10.20, 5.78 and 3.19 % of the constrained variance. Axis 4 is excluded from Fig. 2 because it accounted for < 5 % of the variance and did not reveal any new relationships between plant response and environmental variables. Only environmental variables that exhibited strong relationships (either positive or negative) with the response variables are
presented. Percentage weed cover was not linked to any of the variables and was removed from the model.

Bulb width and seed production per bulb varied the most along the first canonical axis (Fig. 2A). The two environmental factors that varied most along the same axis were total light and CCI, indicating that bulbs were larger and seed production was higher under late-closing canopies (i.e., low CCI), and where total light that was received during the growing season was higher. Soil moisture also varied the most along axis 1 and was positively linked to total leaf width. Because its vector varied in the opposite direction from that of bulb width and seed production vectors along axes 2 and 3 (Fig. 2C), we cannot confirm a positive relationship between soil moisture and these two response variables. Average soil temperature exhibited a weak positive relationship with bulb width (Fig. 2C); in the last week of April, plots with a higher temperature had a higher percentage of leaves in stage 2 (unfolding; \( r = 0.589; P = 0.002; \) data not shown). Soil water content data from the first survey (April 21-22) were discarded due to an error in programming the TDR, but we suspect near-saturation conditions in all plots because of the recent melting of the snow cover.

There was a negative relationship between bulb number and light availability after canopy closure, together with average temperature and its variance (Figs. 2A, 2C); bulb division and survival may be affected by high and fluctuating temperatures under a sparse canopy. In contrast, the vector for the percent litter cover was oriented in the same direction as that for bulb number. Bulb number could be thus considered as being positively linked to percent litter cover. Wild leek phenology index (WLPI) and CCI varied in opposite directions along axes 1 and 3, but not along axis 2. However, variation in these variables along axis 2 was marginal. High soil moisture
seemed to favour a higher WLPI (Figs. 2A, 2B). Soil pH ranged from 4.09 to 6.72. It varied mostly along axis 4 and was uncorrelated with the response variables (data not shown).

Acclimation to light conditions

In order to formally test the two components of the main hypothesis, i.e., that 1) a sparse canopy and 2) late canopy closure would favour wild leek growth, the relationships between bulb width and total light availability for the growing season, daily light availability after canopy closure, and CCI were tested (Fig. 3). Bulb width is the best indicator of annual growth of individual wild leek plants and was thus selected as the main response variable. Using the bulb width ratio of years 2013/2012 yielded similar results, but because of missing values in 2012, we preferred performing the analysis with the complete data set of 2013. Total light reaching the understory during the growing season explained 62% of bulb-width variance (Fig. 3A). The two sub-components that influenced the most the total light that was received throughout the wild leek growing season (19 April to 15 June) are presented in Figs. 3B and C. Bulbs appeared to be unaffected by light level after canopy closure (Fig. 3B), but grew larger under a late-closing canopy (Fig. 3C). Photosynthetic rates also could have been affected by the proportion of total light that was direct rather than diffuse (percentage of direct light). There was a marginally non-significant trend towards a positive relationship between bulb width and percentage of direct light during the growing season (not shown; \( r = 0.33; P = 0.08 \)), but there was no relationship between bulb width and percentage of direct light after canopy closure (not shown; \( r = 0.14; P = 0.47 \)).

Of the plots within canopy gaps, only one benefited from higher light conditions after canopy closure. Plots in the tree plantations received more light, grew larger bulbs, and their canopy closed later, when compared to the plots located in natural stands. Wild leek phenology
index and canopy closure index were negatively correlated (Fig. 4); wild leek in plots under a late-closing canopy (low CCI) had a longer active period before senescence (high WLPI). This relationship was significant for the two years that these variables were recorded. We also have reported a strong correlation between WLPI and CCI in natural populations of wild leeks ($r^2 = 0.604; P = 0.008$; Dion, 2015). RDA displayed this strong negative relationship between WLPI and CCI (Figs. 2A, 2B).

RDA indicated that percent litter cover exerted effects that were opposite to those of total light (Fig. 2). To confirm that the observed responses of wild leek growth actually originated from light availability rather than from the presence of a thinner litter layer, we analysed the chlorophyll a/b ratio and the stable carbon isotopic ratio ($\delta^{13}$C). Both ratios were significantly correlated with total light and mean bulb width (Fig. 5). Plants growing under high light conditions produced leaves with higher chl a/b and higher $\delta^{13}$C ratios, indicating acclimation to higher light conditions and enhanced RUBISCO activity over the entire growing season. Consequently, plants with higher chl a/b and $\delta^{13}$C ratios produced larger bulbs. This response was not due to a thinner litter layer, but most likely occurred in response to light availability.

**Discussion**

**Light**

Greater light levels reaching the soil during the growing season translated into better growth of the spring ephemeral. Canopy phenology is the sub-component of the light environment that explains most of the differences in bulb growth and seed production. Delayed canopy closure, as was encountered under *Carya, Fraxinus, Juglans, Quercus* or *Tilia* canopies (Supplementary Table S1), resulted in a longer period of high light conditions and allowed more
carbon accumulation in the bulb and greater investment in seed production. This enhanced productivity was most likely a result of the acclimation of spring ephemeral phenology to canopy phenology; leaf senescence was delayed under a late bud-bursting canopy. This is consistent with previous observations on another spring ephemeral, the katakuri *Erythronium japonicum* Decaisne, which senesced sooner under artificial early canopy closure (Kim et al., 2015). Other studies conducted on the influence of the light environment on wild leek growth did not consider tree phenology, which could explain why no effect of light was detected (Vasseur and Gagnon, 1994; Delagrange et al., 2013). The effect of tree leaf phenology on the growth of forest herbs has been seldom studied, but Routhier and Lapointe (2002) found that *T. erectum* growth and reproduction were enhanced when there was a longer period of high light conditions prior to canopy closure. Further, it should be considered that southern Québec is located close to the northern limits of wild leek’s geographic range (NatureServe, 2015). The time period between snowmelt and canopy closure is shortened in sugar maple forests that are growing at more northern latitudes (Routhier and Lapointe, 2002). This may contribute to explaining the critical importance of canopy phenology for spring ephemeral growth that we recorded in the present study.

Light has been reported to affect other early summer forest herbs, even without considering tree phenology. For example, Naud et al. (2010) found a significant positive effect of light availability after canopy closure on the growth of the forest herbs black cohosh (*Actaea racemosa* L.), wild ginger (*Asarum canadense* L.), and bloodroot (*Sanguinaria canadensis* L.), but not on blue cohosh (*Caulophyllum thalictroides* [L.] Michaux). Ida and Kudo (2008) reported an effect of shading on reproduction and carbon allocation in *Trillium apetalon* Makino. In the present study, variation in the chl a/b ratio and in δ¹³C as a function of total light reaching the
plot during the season confirmed that wild leek did acclimate to the light conditions and that mean photosynthetic rates increased with light availability, as would be expected for leaves that are acclimated to higher light conditions (Nobel, 1991). Light acclimation most likely took place following a first growing season under the new growth conditions, as mature wild leek do not acclimate their leaves within a single season (Bernatchez et al., 2013; Dion et al., 2016). This acclimation to light availability could explain the increased bulb size that was reported for plots receiving more light throughout the growing season, along with delayed leaf senescence in these plots. Phenotypic plasticity, such as that reported here for leaf phenology and photosynthetic capacity, could help promote relatively high fitness across various environments without high maintenance costs (Griffith and Sultan, 2012).

Gaps in the canopy did not have the expected positive effect on light availability. Of the five plots that were located within a gap, four plots had light conditions similar to the other plots that were located under closed canopies. Those four plots were positioned directly in the centre of the gap. Given that the sun does not pass at the zenith at a latitude of 44° N, these plots did not receive more direct light. Indeed, according to Canham (1988b), the point receiving the most light within a 10-metre diameter gap located at these latitudes would actually be located 5 m outside the vertical projection of the gap. Moreover, the percentage of total light transmitted as direct light only marginally affected growth of wild leek. While we would expect a shade-avoiding herb to respond more to direct than to diffuse light, most of the light that is available in temperate deciduous forests during spring is indeed direct (Brantley and Young, 2009). Spring ephemerals may be light-saturated during most of their growing season, until the canopy closes. Hence, there is an absence of a significant relationship between wild leek growth and percentage of direct light. The fifth gap plot received much more light after canopy closure than did the other
plots, but this light surplus did not translate into enhanced bulb width. There was no reason to remove this apparent outlier from the correlation that is presented in Fig. 3B, but doing so would change the relationship from one that was non-significant to one that was weak but significant ($r^2 = 0.156, P = 0.034$). This result would suggest that increased light availability under a sparse canopy could have a positive and significant, albeit marginal, effect on wild leek growth. One would not expect the growth of a spring ephemeral to be influenced by the amount of light passing through the canopy following its closure. However, we have recently shown that modulating light during leaf senescence in wild leek influences the photosynthetic rates that are recorded in the green sections of the leaf, as well as final bulb size (Dion et al., 2016). The light conditions during leaf senescence can thus influence the total amount of C that is fixed and, therefore, the amount of C that accumulates in the bulb.

Other abiotic factors

Temperature has been previously identified as a critical factor triggering leaf unfolding, flowering and senescence of spring ephemerals (Lapointe, 2001; Yoshie, 2008; Bernatchez and Lapointe, 2012) and other early summer forest herbs (Dahlgren et al., 2007; Jochum et al., 2007), yet canopy phenology had a stronger influence than did temperature on the overall length of the epigeous period during the present study, according to the RDA (Fig. 2). However, the light environment could have influenced soil temperature. The mean and variance of soil temperature were positively linked to light levels that were transmitted after canopy closure, which is mostly a function of canopy density. A dense forest canopy is known to stabilize air and soil temperature, and soil moisture (Morecroft et al., 1998), which could benefit spring ephemerals (Diaci et al., 2012).
Soil moisture emerged as the most important environmental factor explaining total leaf width variation and ramet demography of wild leek in an experiment that was conducted by Vasseur and Gagnon (1994). We observed a similar positive link between soil moisture and leaf width, but not with the number of bulbs or the size of bulbs. Another spring ephemeral, *E. japonicum*, exhibited a reduced photosynthetic rate in a dry environment under an open canopy, a response that could be overcome by increasing the relative humidity of the air (Sawada et al., 1997). Likewise, Axmanova et al. (2011) reported a small, but significant positive influence of soil moisture levels on overall herb layer productivity in central European oak forests. In our experiment, the relationship between soil moisture and the wild leek phenology index was unexpected, but could be explained by a 23-day period of sunny conditions from 17 April to 9 May 2013. Only 9.6 mm and 19 mm of rain fell respectively in the Lower Laurentian and Estrie regions during this period (Environment Canada, 2013), at the end of which plants from some plots began to show early signs of senescence. Similarly, Tessier (2008) reported reduced leaf longevity for *T. erectum* during a dry summer.

The negative relationship between percent litter cover and total light reaching the understory was expected, since a denser canopy should shed more leaves (Lebret et al., 2001). Although the chl a/b ratio and δ^{13}C analyses confirmed that the direct effect of canopy type on wild leek individual growth mostly resulted from differences in light availability, litter could still exert an important effect on spring ephemeral growth through its impact on soil temperature. The present results show that litter reduced the mean and variance of soil temperature. Indeed, litter layer thickness is known to stabilize soil temperature, to isolate the soil from the air, and to intercept solar radiation (Facelli and Pickett, 1991). This buffering resulted in greater survival in plots with a higher litter cover. Baltzinger et al. (2012) also reported greater leafing success for
the spring ephemeral *Anemone nemorosa* L. (wood anemone) when litter was present. They also reported greater rhizome growth for the wood anemone in plots with litter, whereas we found that wild leek growth increased with increased soil temperatures. Higher soil temperatures in early spring would lengthen the growing season, thereby benefiting spring ephemerals, but higher soil temperatures later in the season might cause mortality.

**Concluding remarks**

A longer period before spring canopy closure, combined with a dense canopy during summer, significantly improved growth of wild leek in transplant plots. This response is consistent with results that have been amassed from the few other studies that have been performed on spring ephemeral plant phenology. More specific research is still needed to differentiate the influence of various tree species, but we can already state that late bud-bursting genera, such as *Fraxinus, Juglans, Quercus* and *Tilia*, provide an optimal light environment for wild leek. Specific canopy composition could be less important in natural populations of this spring ephemeral, where other environmental factors, such as soil moisture, nutrient availability or temperature, can vary greatly among populations. Further research is required to assess the importance of litter composition and abundance, and soil moisture for spring ephemeral growth. Nevertheless, this study confirms the plasticity of wild leek leaf phenology, highlights its capacity to acclimate to changes in light availability from one year to the next, and emphasizes the importance of canopy phenology and total light availability for the growth and reproduction of spring ephemerals.
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Litterature cited


Dion, P.-P. 2015. Caractérisation des conditions de culture propices à l’ail des bois en système agroforestier. M.Sc. Thesis, Department of Biology, Laval University, Québec, QC.


Lavallée, R. 1978. Quelques aspects de la biologie de l’ail des bois. Université Laval, Québec, QC.


**Figure captions**

Figure 1. Leaf phenological stages of wild leek that were used in the calculation of WLPI. 1 = leaf emergence; 2 = leaf unfolding; 3 = fully expanded leaves; 4 = beginning of senescence with < 25 % of leaf area senesced; 5 = advanced senescence with > 25 % leaf area senesced; 6 = fully senesced leaves.

Figure 2. Redundancy analysis of wild leek growth variables (grey, dashed lines) constrained by environmental variables (black, full lines). Each dot represents a transplant plot. AT = average soil temperature; BN = bulb number per plot; BW = bulb width per plant; CCI = canopy closure index; LAC = light availability after canopy closure; Lit = litter cover; M = soil moisture; pH = soil water pH; S = seed number per plant; TL = total light during the growing season; TLW = total leaf width per plant; VT = mean daily variance of soil temperature; WLPI = wild leek phenology index.

Figure 3. Linear response of mean bulb width versus a) total light availability during the growing season and two light parameters that influence total light availability in the transplant plots, i.e., b) light after canopy closure and c) the canopy closure index. Light after canopy closure is estimated from hemispheric photographs and includes both direct and diffuse light.

Figure 4. Linear relationship between Wild Leek Phenology Index (WLPI) and Canopy Closure Index (CCI) in transplant plots. Phenological observations were recorded in 2012 (A) and 2013 (B). The two data sets were analysed separately, because the surveys were not done over the exact same periods, and this influenced WLPI and CCI values.
Figure 5. Chlorophyll a/b ratio (A, B) and stable carbon isotopic composition (δ^{13}C; C, D)

exhibited a significant linear relationship with total light reaching the plot during the growing
season (A, C) and the mean bulb width (B, D).
Figure 1
Figure 2
Figure 3

A. Mean bulb width (cm) vs. total light in growing season (mols photons m$^{-2}$).

\[ y = 8.27 \times 10^{-6}x + 0.74 \]
\[ r^2 = 0.62, P < 0.01 \]

B. Light after canopy closure (mols photons m$^{-2}$ d$^{-1}$).

\[ y = 3.49 \times 10^{-4}x + 1.04 \]
\[ r^2 = 0.08, P = 0.13 \]

C. Canopy closure index (CCI).

\[ y = -1.98 \times 10^{-1}x + 1.78 \]
\[ r^2 = 0.54, P < 0.01 \]
Figure 4

**A**

\[ y = -0.29x + 37.53 \]
\[ r^2 = 0.37, P < 0.01 \]

**B**

\[ y = -0.45x + 44.96 \]
\[ r^2 = 0.42, P < 0.01 \]