Thermal acclimation of leaf respiration as a way to reduce source-sink imbalance at low temperature in *Erythronium americanum*, a spring ephemeral

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Thermal acclimation of leaf respiration as a way to reduce source-sink imbalance at low temperature in *Erythronium americanum*, a spring ephemeral

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Abstract

Many spring geophytes exhibit greater growth at colder than at warmer temperatures. Previous studies have suggested that there is less disequilibrium between source and sink activity at low temperature, which delays leaf senescence and leads to higher accumulation of biomass in the perennial organ. We hypothesize that dark respiration acclimates to temperature at both leaf and bulb level, mainly via the alternative pathway, as a way to reduce source-sink imbalance. *Erythronium americanum* was grown under three temperature regimes, 8/6 °C, 12/8 °C and 18/14 °C (day/night). Plant respiratory rates were measured at both growth and common temperature to determine whether differences were due to direct effects of temperature on respiratory rates or to acclimation. Leaf dark respiration exhibited homeostasis, which together with lower assimilation at low growth temperature, most likely reduced the quantity of C available for translocation to the bulb. No temperature acclimation was visible at the sink level. However, bulb total respiration varied through time, suggesting potential stimulation of bulb respiration as sink limitation builds up. In conclusion, acclimation of respiration at the leaf level could partly explain the better equilibrium between source and sink activity in low-temperature grown plants, whereas bulb respiration responds to source-sink imbalance.

Keywords: source–sink relationship, thermal acclimation, sink limitation, *Erythronium americanum*
Introduction

Temperature affects protein synthesis and enzyme activity, which in turn influence the rates of metabolic reactions, such as photosynthesis and respiration (Raison 1980; Atkin et al. 2005b). However, some plants adjust their metabolic rates to partly compensate for the negative impact of changing conditions in an attempt to maintain their growth rate over a broader range of temperatures. This process is called acclimation (Levitt 1972; Berry and Bjorkman 1980). In many instances, the acclimation process can be extended to achieve homeostasis, i.e., where rates of metabolic processes are identical in plants that are grown at contrasting temperatures when measured at their respective growth temperatures (Atkin et al. 2000b). Homeostasis has been demonstrated in many global warming studies (Atkin et al. 2000a; Atkin and Tjoelker 2003), where differences in growth temperatures are not too large, thereby allowing for complete adjustment of the different metabolic rates. Indeed, it has been reported that both leaf total dark respiration (leaf R_T) and net assimilation (A) can acclimate to the extent that the leaf R_T / A quotient remains fairly stable once the leaves have adjusted to the new growth condition (Dewar et al. 1999; Loveys et al. 2003).

Both leaf R_T and photosynthetic rates respond in a substrate-dependent manner. While photosynthesis is feedback-inhibited by accumulation of carbohydrates within the leaf (Foyer et al. 1990; Goldschmidt and Huber 1992; Strand et al. 1997), R_T may be stimulated by an increase in substrate availability (Atkin and Tjoelker 2003). One of the factors that can stimulate leaf carbohydrate accumulation is a reduced rate of translocation between leaves and sink organs (Krapp and Stitt 1995; Ainsworth and Bush 2011). Reduction in C translocation rates has been demonstrated in winter wheat (Triticum aestivum L.) and sunflower plants (Helianthus annuus L.) that developed at lower temperatures compared to rates that were measured in warm-grown plants (Paul et al. 1990; Leonardos et al. 2003). Yet, not all species exhibit such reductions during cold acclimation. Oilseed rape (Brassica napus L.) plants that were grown at 13 °C exhibited greater C translocation rates than those that were grown at 30 °C (Paul et al. 1990) while spring crocus
(Crocus vernus [L.] Hill) that was grown at 12 °C and 18 °C exhibited similar rates of translocation from leaves to corms, early in the season (Badri et al. 2007). Despite potential metabolic adjustment during the acclimation process, carbohydrate accumulation could still take place under low temperature, which could increase the leaf R\textsubscript{T}/A quotient (Atkin et al. 2005\textsuperscript{a}; Campbell et al. 2007).

Another condition where carbohydrates could accumulate within the leaves is under conditions of sink-limited growth (Basu et al. 1999; Hoch et al. 2002). Under such conditions, stimulating leaf R\textsubscript{T} could reduce source-sink imbalances, which are known to induce early leaf senescence through feedback inhibition (Gandin et al. 2009). Such increases in respiration could be due, in part, to increased electron flow to the alternative respiratory pathway (Vanlerberghe and McIntosh 1992; González-Meler et al. 1999; Florez-Sarasa et al. 2007). One of the potential roles for the alternative respiratory pathway is the consumption of excess carbohydrates that are not used for energy production, growth and maintenance processes in tissues, namely the “energy overflow” hypothesis proposed by Lambers (1982). Indeed, several studies have reported results that were consistent with the hypothesis that the alternative pathway acts to burn excess carbohydrate under many stress conditions, namely drought and light stress (Giraud et al. 2008), low N availability (Noguchi and Terashima 2006), and macronutrient stress (Sieger et al. 2005).

Gandin et al. (2009) have previously shown that the capacity of the alternative pathway (R\textsubscript{alt}) was strongly stimulated in the bulb of spring ephemerals under sink-limited conditions that were caused by plant exposure to elevated CO\textsubscript{2} concentrations. Although the non-energy conserving nature of the alternative pathway would be expected to negatively affect plant growth, its positive effects in the maintenance of metabolic and signalling homeostasis might more than offset its negative effects (Vanlerberghe 2013).

In spring ephemerals, the growth of the perennial organ (bulb or corm, according to the species) was shown to be higher at low temperature compared to that recorded at warmer temperatures (Lapointe and Lerat 2006; Badri et al. 2007; Lundmark et al. 2009; Gandin et al.
2011; Bernatchez and Lapointe 2012). As the new bulb/corm accumulates carbohydrates, sink limitation builds up, inducing feedback inhibition of photosynthesis and eventually leaf senescence (Badri et al. 2007; Gandin et al. 2011). It has been suggested that an enhanced bulb/corm growth at low temperature is due to the capacity of the plant to maintain a better equilibrium between source and sink activities over time (Gandin et al. 2011). Therefore, sink limitation and consequent feedback inhibition on photosynthetic activity is postponed at lower temperatures resulting in a longer leaf life duration and a longer period of bulb/corm growth. However, a number of questions remain pending. Could this equilibrium be linked not only to reduced assimilation, but also to increased respiration at low growth temperature? Could $R_{alt}$ be involved in the modulation of leaf or bulb $R_T$ as a response to temperature? Could respiration be modulated both at the leaf and at the bulb level to more efficiently balance the amount of C that is translocated from leaf to sink and the amount of C that is used at the sink level? Finally, could leaf or bulb $R_T$ be modulated through time as sink limitation builds up?

The main objective of the present study was thus to determine if both $R_T$ and $R_{alt}$ are stimulated at low growth temperature in spring ephemerals to better adjust the C that is required for growth with the C available from assimilation. If so, does it occur in the leaf, in the bulb or in both organs? We assessed the relative contribution of the capacity of the cytochrome ($R_{cyt}$) and alternative ($R_{alt}$) pathways as a function of growth temperature throughout the season in both leaf and bulb of the spring ephemeral, yellow trout lily (Erythronium americanum Ker-Gawl.). Plants were grown under three temperature regimes, i.e., 8/6 °C, 12/8 °C and 18/14 °C (day/night). In forests nearby Quebec City, the 12/8 °C temperature regime represents the mean day and night temperatures during the early growth period of this species (first two weeks of May), while the highest growth temperature represents mean day and night temperatures at the end of its growing period (first two weeks of June). The 12/8 °C temperature regime seems not to be the optimal growth temperature since a better bulb growth was previously observed at 8/6 °C than at 12/8 °C by Gandin et al. (2011). Respiratory rates were measured at both growth temperatures and at a...
common temperature (12 °C) to discriminate the effect of the measured temperature from the acclimation process to growth temperature. Soluble sugar concentrations were also recorded in both leaf and bulb to determine if respiratory rates are modulated as a function of substrate availability.

**Material and methods**

**Plant material and experimental design**

Bulbs of *E. americanum* were collected from a maple forest near Saint-Augustin-de-Desmaures (QC, Canada; 46°48’N, 71°23’W) in September 2012. About 650 bulbs of similar diameter (6-8 mm) were selected and planted individually in 10-cm diameter plastic pots containing Turface (calcined clay granules, Applied Industrial Materials Corp., Buffalo Grove, IL, USA) as substrate. Pots were then placed in a cold room (4-5 °C) for five months of cold stratification. Substrate moisture content was checked weekly and pots were watered when the top 5 cm of Turface had dried. At the end of February, all pots were moved to a growth chamber that was set at 8/6 °C (day/night) in darkness for about one week of acclimation. Thereafter, about 150 pots were randomly transferred to each of three growth chambers (PGW36, Conviron Inc., Winnipeg, MB, Canada) under the following light conditions: photoperiod of 14 h and a photosynthetic photon flux density (PPFD) of 300 µmol. m⁻². s⁻¹. The temperature regimes that were used in the experiment were based upon a study by Gandin et al. (2009); three regimes were tested: 8/6 °C (day/night), 12/8 °C, and 18/14 °C, with relative humidities (RH) of 50 %, 65 %, and 75 %, respectively. RH was modulated as a function of temperature to maintain a constant vapour pressure deficit (VPD) among growth chambers. Plants were watered regularly and fertilised weekly with 10 % Hoagland’s solution for optimal plant growth (Lapointe and Lerat 2006).

**Plant phenological stages**
Plant phenology was recorded throughout the growing season (Fig. 1) and was organised according to well-known phenological periods: (i) leaf expansion, hereafter referred to as period I (leaf sprouting and unfolding; old bulb acting as a source); (ii) green leaf period, which is divided into period II (leaf expansion completed; continued shrinkage of the old bulb continues; the new bulb is visible at the end of this period) and III (most new bulb growth occurs during this period); (iii) leaf senescence period, which is divided into period IV (beginning of leaf senescence up to mid-senescence; leaf changes from exhibiting a yellow tip to about half-yellow; bulb biomass is no longer increasing) and V (mid- to complete leaf senescence; bulb enters into dormancy). In the present study, the different variables were measured at specific sampling stages (identified as T1 to T5) during the season, which covered the different phenological stages of both leaf and bulb (Fig. 1).

**Leaf assimilation measurements**

Net assimilation rates (A) were measured using a portable photosynthesis system (Li-Cor 6400, Li-Cor Inc. Lincoln, NE, USA). Light was set at 300 µmol. m\(^{-2}\). s\(^{-1}\), which was similar to light levels under growth conditions, and airflow was set at 200 µmol. s\(^{-1}\). Temperature and relative humidity conditions were similar to those recorded in the growth chambers. Measurements were performed on five plants (i.e., five leaves) in each growth chamber. All measurements took place on leaves that had been exposed to at least 2 h of daytime lighting in the growth chambers.

**Leaf and bulb respiration measurements**

One hundred mg of fresh leaf discs (6 mm in diameter) and bulb pieces (4×4×1 mm) were sampled from five plants per growth chamber per sampling stage, rinsed and vacuum infiltrated in a syringe with reaction medium. The medium contained 100 mM mannitol, 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 10 mM MES (2-(N-morpholino)ethanesulfonic acid, pH 6.6) and 0.2 mM CaCl\(_2\), according to the method described by Jolivet et al. (1990). For
the samples that were harvested at 50% of leaf senescence (T5), a 50:50 mixture of green and yellow leaf sections were used. The fragments were transferred to a dissolved O₂ electrode incubation chamber (Rank Brothers Ltd., Cambridge, UK). O₂ uptake was measured with this Clark-type polarographic electrode in 4 mL of air-saturated reaction medium under the day growth temperatures (i.e., 8 °C, 12 °C and 18 °C, respectively), and at a common temperature of 12 °C. Aqueous KCN (1 mM) and salicylhydroxamic acid (SHAM, 10 mM), which was dissolved in methoxy-ethanol, were used as inhibitors of the cytochrome pathway and of the alternative pathway, respectively. The O₂ electrode chamber was covered with aluminium foil during leaf measurement, to ensure that leaf dark respiratory rates were being measured.

For both organs, total respiratory rate (Rₜ) was measured at first without any inhibitors. The capacity of the alternative pathway (Rₐₜₕ) was determined using the equation: Rₐₜₕ = Rₜ+KCN − Rₑ₂ₕ, where Rₜ+KCN is the respiratory rate that was measured after the addition of the inhibitor KCN, and Rₑ₂ₕ (residual respiratory rate) was measured by adding the second inhibitor SHAM. The capacity of the cytochrome pathway (Rₑₕ) was determined by the equation: Rₑₕ = Rₜ+SHAM − Rₑ₂ₕ, where Rₜ+SHAM is the respiratory rate that was measured when the inhibitor SHAM was added first.

All leaf gas exchange data (assimilation and respiration measurements) were converted to the same units (µmol O₂ min⁻¹ gDW⁻¹). Given that the number of replicates in the assimilation measurements (n = 5) and the respiration measurements (n = 2 to 4) were unequal, and done on different samples, we used a permutation approach to calculate between 10 and 20 different quotients of leaf dark respiration to net assimilation (leaf Rₜ / A) per growth temperature and phenological stage. All these estimated quotients were used as replicates in the statistical analysis. We considered that there were only 5 replicates when calculating the standard error of the mean in order to avoid under-estimating it.

Non-structural carbohydrate concentrations
For each treatment and at each sampling stage, five plants were harvested 3 hours after the beginning of the light period. Leaves and bulbs were separated and flash-frozen in liquid nitrogen. Plant material was then freeze-dried, weighed and ground into fine powder in a ball mill (Qiagen Inc., Toronto, Canada). Soluble sugars were analysed in both leaf and bulb samples, whereas starch was only analysed in bulb samples, given that leaves in this species do not contain significant quantities of starch (Gandin et al. 2009). Fifty mg of freeze-dried ground material was macerated for 20 minutes in a methanol/chloroform/water solution (MCW 12:5:3, v/v) at 65 °C. After homogenisation using a Polytron (Kinematica, Lucerne, Switzerland), the mixture was centrifuged at 3 500 rpm for 10 minutes at 4 °C. The supernatant was then harvested and stored temporarily on ice. In order to complete the sugars extraction from the remaining pellet, homogenisation and centrifugation were repeated, and the second supernatant was added to the first one. Total soluble sugars were analysed by reaction of 100 µL of the supernatant with 1.5 mL of freshly prepared anthrone solution (Hansen and Møller 1975) in warm water (60 °C) for 20 minutes. After cooling, the absorbance was determined at 620 nm with a spectrophotometer (Beckman DU640, Beckman Coulter, USA). Glucose was used as standard.

The pellet containing starch was gelatinised in boiling water for 90 minutes, then hydrolysed at 55 °C for 60 minutes in the presence of amylglucosidase (Sigma-Aldrich, St. Louis, MO). After cooling and centrifugation (3 500 rpm for 5 minutes), starch concentration was determined colorimetrically at 415 nm on a glucose-equivalent basis using p-hydroxybenzoic acid hydrazide (Blakeney and Mutton 1980).

**Statistical analysis**

Two-way ANOVAs were carried out to assess both the effect of growth temperature and phenological stage on non-structural carbohydrate concentrations in the leaf and bulb, leaf and bulb respiration measurements ($R_T$, $R_{cyt}$ and $R_{alt}$), leaf net assimilation ($A$) and the quotient of $R_T$/A in the leaf. These analyses were followed by Tukey HSD tests for multiple comparisons when
main effect or the interaction was statistically significant. Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC) and graphs were generated with Prism 6.0 (Graphpad Software Inc., La Jolla, CA).

Results

*Plant phenology*

Growth duration of *E. americanum* decreased as growth temperature increased, lasting 73 days, 56 days and 43 days for plants grown under the 8/6 °C, 12/8 °C and 18/14 °C regimes, respectively (Fig. 1). Similar responses were exhibited for the individual phenological stages (periods I to V). Leaf expansion (period I) lasted 9 days, 7 days and 5 days for plants grown at 8/6 °C, 12/8 °C and 18/14 °C, respectively. New bulb growth started within the core of the old bulb at T2 (period III of the green-leaf period), which occurred at days 19, 15 and 11 for plants grown at 8/6 °C, 12/8 °C and 18/14 °C, respectively (Fig. 1). Initiation of leaf senescence (T4), which also corresponds to the termination of new bulb growth, was recorded at days 47, 34 and 24 for plants grown at 8/6 °C, 12/8 °C and 18/14 °C, respectively. Leaf senescence (periods IV + V) also lasted longer under the coolest growth temperature regime (Fig. 1).

*Leaf dark respiratory rates*

Leaf $R_T$ was fairly constant throughout the growing season, except in senescing leaves (T5) where it decreased (Fig. 2A and Table 1). $R_{cyt}$ was highest at the beginning of the growth season when the leaf was expanding, and gradually decreased with time until senescence (Fig. 2B and Table 1). $R_{alt}$ remained fairly constant over most of the season (Fig. 2C and Table 1), except during leaf senescence where it declined. Plants grown at the three different growth temperatures exhibited similar $R_T$ and $R_{alt}$ when measured at their respective growth temperatures (Figs. 2A, 2C and Table 1), whereas $R_{cyt}$ was generally higher in plants grown at the highest growth temperature (Fig. 2B and Table 1).
When respiratory rates were measured at a common temperature (12 °C), $R_T$ was stimulated in cold grown plants, but not through an increase in the capacity of either the cytochrome or the alternative respiratory pathway (Figs. 2D, 2E and Table 1). An enhanced effect of low growth temperature on $R_{alt}$ was observed only at T2 (Fig. 2F).

**Leaf A and $R_T/A$ quotient**

Net assimilation (A) increased quickly from leaf unfolding (T1) to reach maximum rates at T3, after which A decreased continuously until T5 (Fig. 3A and Table 1). Leaf A was significantly higher in plants grown at warmer temperatures than in those grown at the cooler treatment (Fig. 3A and Table 1). The leaf $R_T/A$ quotient was high early in the season in plants regardless of their growth temperature regime, then decreased to reach a minimum at either T3 (8/6 °C and 18/14 °C) or T5 (12/8 °C). The $R_T/A$ quotient was significantly higher in plants grown at 8/6°C compared to the other treatments. This was observed throughout the growing season, except at stage T4 where the quotients were similar among growth temperatures (Fig. 3B and Table 1). For plants that were grown at 8/6 °C, leaf $R_T$ represented as much as 37 % of A early in the season, and reached as low as 9 % at T3. The decrease of the leaf $R_T/A$ quotient throughout the season was more moderate at the two warmer growth temperatures, from 16 % to 7 % at 12/8 °C and from 17 % to 6 % at 18/14 °C.

**Bulb respiratory rates**

Bulb respiratory rates are presented on a starch-free dry-mass basis (µmol O₂.min⁻¹.g starch-free DW⁻¹). This allowed us to gain a clearer picture of the changes in respiratory rates as a function of metabolite concentrations in the soluble fraction of the cells, given that starch concentrations can reach very high values towards the end of the season (Gandin et al. 2011).

As growth temperature × stage interactions were significant for each bulb respiration variable measured at the growth temperature, we cannot describe general patterns that apply to all three
growth temperature regimes (Table 1). At the coolest growth temperature, $R_T$ did not differ through time; whereas $R_{cyt}$ was lower early and late in the season (T1 and T5), $R_{alt}$ was lower only at T2 (Figs. 4A, 4B, 4C and Table 1). In plants that were grown at 12/8 °C, $R_T$ was lowest at T2 and increased from T2 to T4 where it reached a maximum. In contrast, $R_{cyt}$ did not differ through time, whereas $R_{alt}$ was lowest at T3 and T5 (Figs. 4A, 4B, 4C and Table 1). In plants that were grown under the warmest temperature regime, $R_T$ was also lowest at T2 and increased thereafter to reach its maximum at T4. Both $R_{cyt}$ and $R_{alt}$ exhibited a similar pattern as $R_T$ that is, increasing with time from T2 to T4 (Figs. 4A, 4B, 4C and Table 1). In general, all three respiratory variables were higher in plants grown at the two warmer temperatures, although there were some inversions at specific phenological stages. The most obvious one was at T2, where both $R_T$ and $R_{cyt}$ were higher in plants that were grown at the coolest temperature.

When measured at a common temperature (12 °C), growth temperature $\times$ stage interactions were also significant for all variables (Table 1). Differences among plants grown under different temperature regimes were less frequent than when respiratory rates were measured at their respective growth temperatures. $R_T$ and $R_{cyt}$ only differed early and late in the season (T1 and T5), where plants that were grown at the warmest temperature exhibited the highest values (Figs. 4D, 4E and Table 1). $R_{alt}$ was enhanced under the 12/8 °C regime, except at T5, where rates were highest in plants grown at the coolest temperature (Figs. 4F and Table 1).

**Soluble sugar concentrations**

Concentrations of soluble sugars (SS) increased gradually in leaves during the growing season at all three growth temperatures (Fig. 5A and Table 1), and sink limitation appeared to build up earlier at warmer temperatures. Indeed, maximum SS concentrations were recorded a few days prior to leaf senescence (T3) for plants that were grown at 18/14 °C, at the beginning of leaf senescence (T4) for those that were grown at 12/8 °C, and only at mid-senescence (T5) for those that were grown at 8/6 °C. These changes through time represented a two-fold increase for the 8/6
°C and 12/8 °C grown plants, and a 1.5-fold increase for the 18/14 °C grown plants. For most of
the season, leaves of plants that were grown at the lower temperature contained higher SS
compared to those grown at higher temperatures. This was particularly obvious during the period
of leaf senescence (T4 and T5), where SS concentrations increased as growth temperature
decreased.

SS concentrations in the bulb are presented as a function of starch-free dry mass (mg. g starch-
free DW⁻¹), as was the case for bulb respiratory rates. At all three growth temperatures, SS
concentrations remained fairly constant while the old bulb was transferring C towards the leaf (T1
and T2); SS then increased to reach a maximum at T3, i.e., when the new bulb was growing and
accumulating starch (Fig. 5B and Table 1). However, these changes were not significant in plants
grown at the lowest temperature, given that their SS concentrations were also higher, early in the
season, than in plants grown at warmer temperatures. During leaf senescence, when the bulb was
no longer increasing in size, bulb SS concentrations declined about three-fold for plants that were
grown at 8/6 °C and 12/8 °C, and about two-fold for those that were grown at 18/14 °C.
Therefore, bulb SS concentrations were higher in plants that were grown at higher temperatures at
the onset (T4) and during leaf senescence (T5). Growth temperature modulated SS concentrations
differently in the leaf and bulb: maximum concentrations were not reached at the same
phenological stage, except for the plants grown at the warmest temperature regime. Towards the
end of the season, plants grown at warmer temperatures accumulated more SS in their bulbs, but
less in their leaves compared to plants grown at cooler temperatures.

Discussion

Thermal acclimation of respiration

Leaf Rₜ of E. americanum exhibited homeostasis, which suggests that acclimation occurred in the
leaf in response to growth temperature. Similar results of leaf Rₜ acclimation to changes in
growth temperature have been reported in arctic herb Saxifraga cernua (McNulty and Cummins
1987), arctic-alpine *Ranunculus glacialis* (Arnone and Körner 1997), and many other species (Loveys et al. 2003). In contrast, in *Vigna radiata* leaves and *Glycine max* cotyledons, plants grown at cool or warm conditions exhibited different $R_T$ when measured at their respective growth temperature, whereas no differences in respiration were observed when measured at the same temperature (González-Meler et al. 1999). Indeed, when respiration was measured at a common temperature of 12 °C, plants grown at the coolest temperature had higher leaf $R_T$ than plants grown at warmer temperatures (Fig. 2D) suggesting an improved respiratory capacity to compensate for the slower rates that occur at low temperature. However, it appears that acclimation occurred only in plants grown at 8 °C, as plants grown at either 12/8 °C or 18/14 °C exhibited similar leaf $R_T$ when rates were measured at a common temperature (Fig. 2D). This result is consistent with what has been reported in *Saxifraga cernua* (McNulty and Cummins 1987), where the $R_T$ in cool-grown plants was much higher than in warm-grown plants when measured at an intermediate temperature. Similarly, in the leaves of five temperate ruderal species (Collier and Cummins 1990) and in *Vigna radiata* hypocotyls (González-Meler et al. 1999), $R_T$ of cooler-grown plants was consistently greater than that from warmer-grown plants at any given measurement temperature.

Thermal acclimation of respiration does not seem to occur in the bulb. Firstly, bulb $R_T$ was sometimes lower, sometimes higher in the 8/6 °C plants than in the two other groups of plants, depending on the phenological stage (Fig. 4A) indicating that homeostasis was not reached. Secondly, bulb $R_T$ was very similar among plants grown at the different temperature regimes, when measured at a common temperature (Fig. 4D). The two instances where $R_T$ differed among growth temperatures (T1 and T5) could not be explained by thermal acclimation since one would expect thermal acclimation to reduce $R_T$ in plants grown under higher temperature regime, while we recorded increased $R_T$ values in these plants. There are very few studies reporting respiratory rates of either corm, bulb or tuber as a function of growth temperature; the two we are aware of were done on tissue culture *in vitro*. $R_T$ remains relatively constant in potato callus grown at
different temperatures (8 to 28 °C) when measured at a common temperature (28 °C), although the capacity of the alternative pathway increases exponentially with growth temperature (Hemrika-Wagner et al. 1983). Yamagishi (1998) also reported similar $R_T$ in bulblets of *Lilium japonicum* Thunb. grown at either 20 or 26 °C when measured at a common temperature. Therefore, we conclude that bulb respiration did not acclimate to the different temperature regimes.

Previous work in *E. americanum* has shown that $A$ decreased as growth temperature decreased (Gandin et al. 2011); the same trends were recorded in the current study (Fig. 3A). Leaf $R_T$ acclimation at low growth temperature, associated with low $A$, resulted in high leaf $R_T / A$ quotients at low growth temperature, which suggests that a reduced amount of carbohydrates was available for translocation to the bulb. Despite the reduced amount of C available for translocation, SS remained slightly higher in leaves of plants grown at low temperature than in those of plants grown at the two other temperature regimes, suggesting that not only was there less C available for translocation to the sink, but translocation rates were also most likely slower. Translocation rates have previously been shown to be similar at 12 °C and 18 °C in another spring geophyte (Badri et al. 2007), but it is possible that lower temperatures, such as 8 °C, do slow down translocation. For instance, in winter wheat leaves, a proportionally lower C-export rate has been found at 5 °C than at 20 °C (Leonardos et al. 2003). In summary, temperature acclimation of respiration occurred only at the leaf level and only in the low-temperature-grown plants. This thermal acclimation of respiration combined with reduced assimilation rates at low temperature, further decreased the amount of C available for translocation to the sink. By reducing the amount of C available for translocation, plants grown at low temperature would establish a better equilibrium between source and sink activity, prolonging leaf life duration until feedback inhibition of photosynthesis would induce leaf senescence.

We initially hypothesized that $R_{alt}$ would be involved in the modulation of $R_T$ at both leaf and bulb level as a response to temperature; yet similar low leaf $R_{alt}$ were observed in plants grown at
the different temperature regimes, whereas the growth temperature effect on bulb $R_{alt}$ was not consistent. Similar leaf $R_{alt}$ among growth temperatures suggested that leaf $R_{alt}$ thermally acclimated, but differences were not large enough to be detected when measured under a common temperature. Similar results were observed in white spruce ($Picea glauca$) roots where $R_{alt}$ was not affected by growth temperatures or measurement temperatures between 4 and 18 °C, and represented 23 % of the total capacity of electron transport (Weger and Guy 1991). However, a stimulation of $R_{alt}$ at low growth temperatures has been reported in leaves of the perennial herb $Saxifraga cernua$ (McNulty and Cummins 1987) and of several temperate ruderal species (Collier and Cummins 1990). The fact that growth rate was much less affected by low temperature in $E. americanum$ (Gandin et al. 2011) than in the species cited above might partly explain why leaf $R_{alt}$ was not strongly stimulated at low temperatures. When sink activity is decreased, C accumulates in the leaves where it could stimulate $R_{alt}$. Gandin et al. (2009) have shown that bulb $R_{alt}$ can be strongly stimulated when $E. americanum$ plants were grown under elevated CO$_2$ concentrations (high source activity), indicating that bulb $R_{alt}$ can be modulated in response to source-sink imbalance. In warmer-grown plants, where C translocation was most likely higher than in cool-grown plants, we did not detect a consistent increase in bulb $R_{alt}$. We conclude that phenological stage has a stronger impact on the bulb respiratory rates than growth temperature.

**Respiration as a function of phenological stage**

Numerous studies have shown that plant respiration is modulated during organ development, with higher rates of respiration in young compared to mature tissues (Azcon-Bieto et al. 1983; McDonnell and Farrar 1993; Atkin and Cummins 1994; Armstrong et al. 2006). The rate of respiration is often linearly related to relative growth rates of the tissues, which reflects modulation in the production of energy and carbon skeletons to fulfil the needs for biosynthesis and cellular maintenance (Lambers et al. 1998). In $E. americanum$, leaf $R_T$ was relatively constant throughout the period leading up to senescence (Table 1), which would indicate that most of leaf
growth was already achieved at T1. Nevertheless, leaf $R_{cyt}$ was higher at T1, then decreased to reach a stable level up to the beginning of leaf senescence. Florez-Sarasa et al. (2007) previously demonstrated that growth respiration of *Arabidopsis thaliana* rosette leaves was largely dependent on the activity of the cytochrome pathway, which would suggest that growth processes requiring ATP still occurred at T1 in the leaf of *E. americanum*. We expected bulb $R_{cyt}$ to be high at T2 while cells were actively dividing within the new bulb, but this was not the case, except at the coolest growth temperature (Table 1). At T2, the new bulb was very small; we posit that most respiration originated from the old bulb that was being emptied by then and was less metabolically active.

Unlike leaf $R_T$, bulb $R_T$ exhibited a general increase through time, as the new bulb increased in size and accumulated starch, except at the coolest growth temperature. The current results bring support to the hypothesis that bulb $R_T$ increases as sink limitation builds up through time. Under high CO$_2$, where sink limitation was stronger, the increase in $R_T$ over time was more pronounced than under ambient CO$_2$ (Gandin et al. 2009) also pointing toward a modulation of bulb $R_T$ by source-sink imbalance. Steingröver (1981) reported that $R_{alt}$ was higher during early growth than during extensive SS and biomass accumulation in the taproot of carrot (*Daucus carota* L.). Similarly, $R_{alt}$ decreased during inulin accumulation in storage roots of *Hypochaeris radicata* (Lambers and Van de Dijk 1979). These authors concluded that «'This suggests that sugars are oxidized 'wastefully' only if sugar supply from the shoots is in excess of the amount than can be utilized for energy production, for structural growth or for storage»). In plants where most of the growth of the perennial organ takes place later in the season, long after the perennial organ has been developed, sink limitation is more likely to occur early in the season (Steingröver 1981). On the other hand, in spring ephemerals where growth and storage occur concomitantly, sink limitation is most likely to build up through time as growth slows down. However, regardless of the timing of sink limitation, $R_T$ of the perennial organ appears to be strongly modulated by source-sink balance. The improved balance between source and sink activity in cool-grown plants
of *E. americanum* most likely explains why bulb $R_T$ did not steadily increase with time in these plants compared to those grown at higher temperature.

As the respiratory rates that we measured were completely dependent upon endogenous substrate levels, bulb $R_T$ could also vary with carbohydrate availability (Atkin and Tjoelker 2003), which originated either from C stored in the old bulb or C translocated from the leaf. Changes in bulb $R_T$ and bulb SS concentrations were not always related to each other, given that bulb SS concentrations started decreasing between T3 and T4, while bulb $R_T$ continued to increase up to T4 (Figs. 4A and 5B). At the lowest growth temperature, modulation of bulb SS concentrations and bulb $R_T$ over time did not match one another. Similarly, the reduction in $R_T$ in taproot during carbohydrate storage was not accompanied by a reduction in reducing sugar concentrations in *Hypochaeris radicata* (Lambers and Van de Dijk 1979), nor in carrot (Steingröver 1981). Other researchers have also reported conflicting results between carbohydrate levels within a tissue and either $R_T$ (Atkin et al. 2000b) or $R_{alt}$ (Wang et al. 2011). Taking into account the relative growth rate of the storage organ, and thus its sink strength appears to better explain the modulation of its respiratory rates over time than soluble sugar availability.

In conclusion, we recorded some acclimation of respiration at the leaf level, but only for $R_T$, not for either the capacity of the cytochrome or the alternative pathway of respiration. Low temperature acclimation of leaf $R_T$ might partly contribute to the improved growth of the bulb recorded at low growth temperature in spring ephemerals. Low temperature grown plants modulated their leaf $R_T$ to reach leaf homeostasis; they also exhibited reduced A which lead to higher leaf $R_T / A$ and most likely to reduced quantity of C translocated to the bulb at any given time. This reduced amount of C translocated to the bulb would help maintain source and sink activity in balance for a longer period of time at cooler temperatures, thus extending the duration of the growth period. No temperature acclimation of either $R_T$ or $R_{alt}$ was visible at the sink level, and bulb $R_T$ did not vary in concert with SS concentrations. However, bulb $R_T$ did vary through time at the two warmer temperatures, suggesting that bulb $R_T$ was being stimulated as sink
limitation builds up. At the lowest temperature, bulb $R_T$ remained constant throughout the season, in accordance with the better equilibrium between source and sink activity at that temperature.

Acknowledgements

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photosynthetic and C-export patterns in winter wheat leaves during cold stress and


Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast-


Table 1. Summary of two-way ANOVA on the effects of stage (T1 to T5) and growth temperature (8/6 °C, 12/8 °C and 18/14 °C) on leaf and bulb respiratory rates (R_T, R_cyt and R_alt), leaf net assimilation (A), leaf dark respiration to assimilation ratio (leaf R_T/A), and leaf and bulb soluble sugar (SS) concentrations. Bulb respiratory rates and bulb SS concentrations are presented on a starch-free bulb dry-mass basis. F-values are presented, followed by significance level (*, **, and *** denote a significant difference at P < 0.05, < 0.01, and < 0.001, respectively). Multiple comparisons among temperatures and among stages are also shown. Lowercase letters denote significant differences (P < 0.05) among stages (comparisons in each row), and uppercase letters refer to significant differences (P < 0.05) among temperatures (comparisons in each column). Absence of lowercase letters in rows (stage comparisons) and uppercase letters in columns (temperature comparisons) indicates non-significant differences. GT, growth temperature; MT, measurement temperature.

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¹ Significance level: *, ** and *** denote a significant difference at \( P < 0.05 \), 0.01 and 0.001, respectively.

² Multiple comparisons for Stage effect were shown only when Stage effect was significant, but T °C × Stage interaction effect was not significant.

³ Multiple comparisons for T °C effect were shown only when T °C effect was significant, but T °C × Stage interaction effect was not significant.
Figure Captions

**Figure 1.** Representative illustration of plant phenology of *E. americanum* grown at 8/6 °C, 12/8 °C and 18/14 °C. Shown from left to right: duration of leaf expansion (period I), duration of green leaf (period II and III) and leaf senescence period (period IV and V). Arrows indicate the sampling stages T1 to T5. Numbers indicate the duration (in days) of each stage.

**Figure 2.** Total dark respiratory rate (leaf $R_T$, µmol O$_2$. min$^{-1}$. g DW$^{-1}$, A and D), capacity of the cytochrome pathway (leaf $R_{cyt}$, µmol O$_2$. min$^{-1}$. g DW$^{-1}$, B and E) and capacity of the alternative pathway (leaf $R_{alt}$, µmol O$_2$. min$^{-1}$. g DW$^{-1}$, C and F) in the leaves of plants grown at 8/6 °C (white), 12/8 °C (grey) and 18/14 °C (black) during the green leaf period and leaf senescence (T1 to T5), measured at growth temperature (A, B and C) and a common temperature of 12 °C (D, E and F). Means ± standard error of the mean (SEM) are presented ($n$ = 2 to 4). * and ** denote that the respiratory rates differed for at least two out of the three growth temperatures at $P < 0.05$ and $< 0.01$, respectively, within each stage when Temperature × Stage interaction effect was significant. See Table 1 for results of multiple test comparisons.

**Figure 3.** Leaf net assimilation (leaf $A$, µmol O$_2$. min$^{-1}$. g DW$^{-1}$, A) and quotient of leaf total dark respiration to leaf net assimilation (leaf $R_T / A$ quotient, B) of *E. americanum* grown at 8/6 °C (white), 12/8 °C (grey) and 18/14 °C (black) during the green leaf period and leaf senescence (T1 to T5). Means ± standard error of the mean (SEM) are presented ($n$ = 5). *** denotes that $A$ differed for at least two out of the three growth temperatures at $P < 0.001$ within each stage. See Table 1 for results of multiple test comparisons. Different letters refer to significant differences ($P < 0.05$) among temperatures.

**Figure 4.** Total respiratory rate (bulb $R_T$, µmol O$_2$. min$^{-1}$. g starch-free DW$^{-1}$, A and D), capacity of the cytochrome pathway (bulb $R_{cyt}$, µmol O$_2$. min$^{-1}$. g starch-free DW$^{-1}$, B and E) and capacity of the...
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**Figure 5.** Soluble sugar concentrations in the leaf (mg. g DW$^{-1}$, A) and bulb (mg. g starch-free DW$^{-1}$, B) of *E. americanum* plants grown at 8/6 °C (white), 12/8 °C (grey) and 18/14 °C (black) during the green leaf period and leaf senescence (T1 to T5). Data are expressed as mean ± standard error of the mean (SEM) ($n = 5$). *, **, and *** denote that the respiratory rates differed for at least two out of the three growth temperatures at $P < 0.05$, $< 0.01$, and $< 0.001$, respectively, within each stage. See Table 1 for results of multiple test comparisons.
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Figure 2. Total dark respiratory rate (leaf R_{T}, \mu mol O_2 min^{-1}. g DW^{-1}, A and D), capacity of the cytochrome pathway (leaf R_{cyt}, \mu mol O_2. min^{-1}. g DW^{-1}, B and E) and capacity of the alternative pathway (leaf R_{alt}, \mu mol O_2. min^{-1}. g DW^{-1}, C and F) in the leaf of plants grown at 8/6 °C (white), 12/8 °C (grey) and 18/14 °C (black) during the green leaf period and leaf senescence (T1 to T5), measured at growth temperature (A, B and C) and a common temperature 12 °C (D, E and F). Means ± standard error of the mean (SEM) are presented (n = 2 to 4). * and ** denote that the respiratory rates differed for at least two out of the three growth temperatures at P < 0.05 and < 0.01, respectively, within each stage when Temperature × Stage interaction effect was significant. See Table 1 for results of multiple test comparisons.

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