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MODULATION DE LA FORCE DES SOURCES ET DES PUITS DE CARBONE SUR LA CROISSANCE DU BULBE DE L'ÉRYTHRONE D'AMÉRIQUE, *ERYTHRONIUM AMERICANUM*

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Résumé

La chute de luminosité ainsi que l'augmentation saisonnière des températures sont considérées comme les principaux agents induisant la sénescence des feuilles chez les plantes éphémères printanières des forêts décidues. Cependant ces deux facteurs n'expliquent pas directement les variations interannuelles de croissance de l'organe souterrain. Nous suggérons que la longévité des feuilles des géophytes printanières serait déterminée par les conditions de stockage des sucres de réserve (taille de/durée de croissance de l'organe pérenne, vitesse de stockage) et non que la durée de vie des feuilles déterminerait la taille de l'organe pérenne. Nos résultats chez *Erythronium americanum* suggèrent une entrée en sénescence prématurée de la feuille aux températures élevées lorsque les plantes sont cultivées à 12/8°C ou 18/14°C ; lorsque le bulbe arrête de croître, la feuille est encore photosynthétiquement active et la concentration en nutriments élevée, signifiant une remobilisation non achevée des nutriments. L'enrichissement de l'air en CO_2 augmente l'assimilation nette des plantes, mais n'accélère pas la croissance du bulbe, et donc pas l'accumulation des sucres. La force d'un puits de carbone tel que le bulbe pourrait influencer la longévité de la feuille chez les individus immatures de cette espèce.

Abstract

Reduction in light intensity as well as seasonal increase in temperature are considered the main factors inducing the senescence of the leaves of deciduous forest spring ephemerals. However, these two factors cannot completely explain the interannual variations in belowground organ growth. We suggest that leaf longevity of spring geophytes is determined by carbohydrate storage conditions (size and growth duration of the perennial organ, storage duration) and not that leaf lifespan determine the size of the perennial organ. Our results on *Erythronium americanum* suggest that leaf senescence appears prematurely under the higher temperature regime when plants are cultivated at 12/8°C or 18/14°C; when the bulb stops to grow, the leaf is still photosynthetically active and the nutrient concentration is high, meaning their mobilisation is not achieved. CO_2 air enrichment increases plant net assimilation rate but does not increase bulb growth rate, and thus does not enhance carbohydrate storage. The strength of a sink of carbon such as the bulb might influence leaf longevity in single-leaved individuals of this species.

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À mes parents et grands-parents

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Chapitre 1 - Introduction générale

Croissance et allocation des ressources chez les plantes

La croissance des plantes, comme celle de tous les êtres vivants, est déterminée par deux éléments : le bagage génétique contenu dans les cellules, qui contrôle l'organisation structurale et détermine la fonction des tissus et organes, et l'environnement dans lequel la plante évolue. Ce dernier joue un rôle primordial chez les végétaux car, contrairement aux animaux, c'est lors du développement postembryonnaire que la plante prend forme. Ainsi, pour une même espèce, il est possible de distinguer une variabilité phénotypique des individus (appelée plasticité). Enfin, les plantes sont généralement capables de s'acclimater, physiologiquement, à leur environnement afin de favoriser leur croissance et leur survie (Bazzaz, 1997).

Toutefois, indépendamment du milieu dans lequel les plantes évoluent, ainsi que de leur taille ou leur forme, croissance et échanges suivent les mêmes règles. Une plante est constituée d'un appareil racinaire, le plus souvent souterrain, et d'un appareil caulinaire. Dans un contexte physiologique, le rôle principal de l'appareil racinaire est de puiser l'eau et les minéraux dans le substrat afin d'alimenter les différents organes de la plante. L'appareil caulinaire, quant à lui, peut être très simple - comme c'est le cas de notre sujet d'étude, l'érythrone d'Amérique (*Erythronium americanum)*, puisqu'il n'est constitué que d'une feuille chez les individus immatures (les individus matures en possèdent deux) et ne possède pas de tige discernable à l'œil nu - ou complexe comme chez les espèces arbustives ou arborescentes. Quoiqu'il en soit, une partie, si ce n'est la totalité, de l'appareil caulinaire est capable de faire de la photosynthèse. Les produits de cette photosynthèse sont exportés dans toute la plante via la sève élaborée, dont le flux bidirectionnel est fonction de l'importance du gradient osmotique présent entre les différents organes de la plante. Ces gradients sont liés au fait que certains organes font de la photosynthèse, et donc produisent des sucres, alors que d'autres en sont incapables. On parle alors d'organes puits ou sources

de carbone (C) dépendamment de leur statut. Les organes sources de C chez les plantes sont principalement les feuilles matures. Toutefois les organes de réserve, en début de saison de croissance ou après des dommages importants, les tiges, par exemple chez les cactacées, mais également les pièces florales et les fruits lors de leur premier stade de développement (Bazzaz, Carlson & Harper, 1979; Lebon *et al*., 2005), ou encore les racines aériennes (Torrey & Clarkson, 1975; Benzing *et al.*, 1983) peuvent représenter des sources non négligeables de C chez certaines espèces. Parmi les organes puits de C, on retrouve les organes végétatifs (tiges, racines, jeunes feuilles, bourgeons), les organes reproducteurs (fleurs, fruits) et les organes de réserve (bulbes, rhizomes, cormes, racines ou tubercules). L'allocation des ressources aux différents puits se fait en fonction de leur demande. Plus la demande est élevée, plus la force du puits est importante et plus les ressources qui lui sont allouées sont élevées. Bien entendu, dépendamment du stade phénologique de la plante, les puits et leur force peuvent changer.

Relations sources/puits de C et longévité des plantes

Le phénomène de la sénescence en général et des parties aériennes en particulier, ainsi que ses différentes étapes, ont été très étudiés et font l'objet de nombreux ouvrages de synthèse (Thomas & Stoddart, 1980 ; Leshem *et al.*, 1986 ; Noodén & Leopold 1988 ; Buchanan-Wollaston *et al.*, 2003). Suivant la définition de Medawar (1957), contrairement au phénomène de vieillissement qui réfère à l'effet de processus graduels de dégénérescence liés à l'âge sur le fonctionnement des individus, la sénescence implique les processus causant naturellement la mort de la plante ou d'une de ses parties. La sénescence est un processus actif qui implique l'activation et la désactivation de plusieurs gènes (Noodén *et al*., 1997). Elle est le résultat d'une dégénérescence difficilement quantifiable, réversible (Thomas *et al.* 2003) et coûteuse en énergie, dont le principal retour pour la plante est le recyclage de nombreux composés, tels que les nutriments, via leur translocation des parties sénescentes vers les parties fonctionnelles ou les organes reproducteurs. Deux patrons principaux de sénescence des plantes supérieures sont rencontrés dans la nature : la monocarpie et la polycarpie. La monocarpie est caractérisée par une phase reproductive

simple précédant la mort de l'individu ; c'est le cas des plantes annuelles et bisannuelles et de quelques pérennes. Les plantes polycarpiques réalisent, quant à elles, plusieurs phases reproductives ; c'est le cas de la plupart des herbacées pérennes et des plantes ligneuses. Chez les plantes monocarpiques, il est établi que la sénescence est le résultat d'un épuisement consécutif à la reproduction, la plus grande partie des ressources se trouvant dans la plante étant exportée dans les graines et les fruits. Cette relation de cause à effet entre la reproduction et la sénescence a été confirmée à plusieurs reprises même si des espèces telles qu'*Arabidopsis* ne semblent pas suivre ce patron (Buchanan-Wollaston *et al.*, 2003). Au contraire, chez les plantes polycarpiques, la sénescence des feuilles ne semble pas liée au développement des structures reproductrices, donc les causes de la sénescence d'un individu ou d'une de ses parties pourraient être différentes du type monocarpique. Chez les espèces arborescentes et arbustives, il est reconnu que la chute des feuilles est influencée principalement par un facteur lumineux (diminution de la photopériode). Les plantes herbacées, quant à elles, pourraient être davantage sensibles aux changements de température (Noodén, 1988).

Toutefois, les mécanismes mis en jeu dans l'induction et la progression du processus de sénescence sont encore mal connus (Gan & Amasino, 1997 ; Buchanan-Wollaston *et al.*, 2003 ; Yoshida, 2003 ; Wingler *et al.*, 2006) car la sénescence est un trait grandement plastique. Elle est affectée par un grand nombre de signaux, pouvant être d'origine interne tels que l'âge, le développement reproductif, le niveau hormonal et la génétique, mais aussi externe tels que la concentration en CO_2 de l'air, les conditions d'irradiance, le statut nutritionnel et hydrique, et les stress biotiques et abiotiques. L'altération de ces signaux peut alors retarder (Gan & Amasino, 1995 ; Grbic & Bleeker, 1995 ; Ono *et al.*, 2001) ou induire (Thomas & de Villiers, 1996; Weaver & Amasino, 2001) la sénescence de la plante ou d'une de ses parties. Des études ont également montré qu'il était même possible de l'inverser (Zavaleta-Mancera *et al*., 1999). Une des principales difficultés de l'étude de la sénescence provient du fait que les premiers symptômes visibles (jaunissement) se produisent généralement bien après le début des modifications physiologiques et biochimiques au sein de la plante (Stessman *et al.*, 2002 ; Yoshida, 2003) et que le

processus est graduel. Mais la littérature portant sur la question est de plus en plus variée et les médiateurs les plus en vue depuis quelques temps sont les sucres (Pourtau *et al.*, 2004 ; Parrot *et al*., 2005), même si leurs effets suscitent la controverse (Yoshida, 2003; Wingler, 2005). Leur rôle dans la signalisation moléculaire au cours du développement de la plante, et particulièrement lors du processus de sénescence, est maintenant considéré et de plus en plus accepté (Hensel *et al.*, 1993 ; Rolland *et al.*, 2002).

Longévité des feuilles chez les printanières géophytes

La plupart des plantes forestières sont des plantes pérennes et clonales (Whigham, 2004). Parmi celles-ci, on retrouve les plantes printanières et, en particulier, les éphémères printanières, dont la courte durée de vie des feuilles amène à se questionner sur les facteurs qui induisent une sénescence apparaissant hâtive par rapport à ce qui est observé chez la plupart des herbacées vivaces occupant le même milieu, tels que le trille rouge (*Trillium erectum*), le maïanthème du canada (*Maianthemum canadense*), la smilacine à grappe (*Maianthemum racemosum*) ou encore la sanguinaire du canada (*Sanguinaria canadensis*). Chez les espèces éphémères printanières, la sénescence des parties aériennes a lieu au moment de la fermeture de la canopée (Sparling, 1964 ; Vézina & Grandtner, 1965 ; Taylor & Pearcy, 1976 ; Figure 1.1). Il a alors naturellement été avancé que la chute de luminosité dans le sous-bois était le principal facteur induisant la sénescence des feuilles. Cette conclusion fut appuyée, par la suite, par l'étude de Eickmeier et Schussler (1993) qui rapportait que *Claytonia virginica* présente une acclimatation à l'ombre limitée, laissant supposer que c'est aussi le cas pour les autres plantes éphémères et que cette incapacité à s'acclimater à l'ombre pourrait induire la sénescence des feuilles au moment où le couvert végétal se referme.

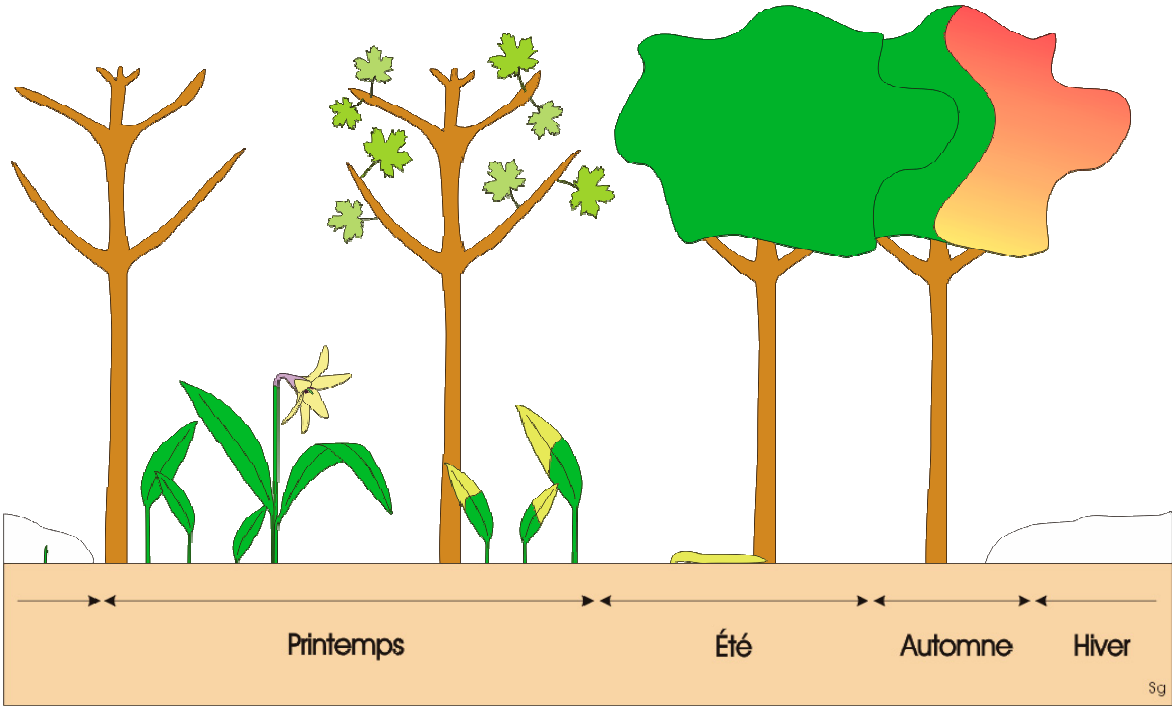

Figure 1.1. Illustration des différents stades phénologiques des parties aériennes de l'érythrone d'Amérique en fonction de la saison. Les individus immatures possèdent une seule feuille, les individus matures en possèdent deux et fleurissent. L'échelle de temps n'est pas respectée pour des fins de clarté.

Toutefois la baisse de luminosité ne semble pas être le signal principal induisant la sénescence, car même en conditions stables de lumière, les plantes présentent une longévité des feuilles limitée. Muller (1978) a montré que l'initiation de la sénescence chez *E. americanum* n'est pas directement corrélée au pourcentage de fermeture du couvert forestier ; les feuilles commençaient à jaunir à 37% de fermeture du couvert forestier la première année de l'étude, mais à 13% l'année suivante, et la sénescence totale eu lieu 16 jours plus tard que la fermeture complète du couvert forestier la première année, mais seulement 6 jours plus tard la deuxième. Il a par contre remarqué que la température au cours du printemps avait une influence sur la phénologie d'*E. americanum*, une hypothèse qui avait été proposée déjà quelques années plus tôt par Vézina et Grandtner (1965).

Des recherches ont alors mené à une étude plus approfondie de l'influence de la température de l'air sur la croissance des plantes éphémères (Risser & Cottam, 1967 ; Yoshie & Fukuda, 1994). Les résultats qui en découlèrent furent similaires à ceux trouvés chez des plantes de culture, c'est-à-dire que la longévité des feuilles y est sensible et négativement corrélée (Cutforth & Shaykewich, 1989 ; Turc & Lecoeur, 1997). L'idée qui en découle est que les feuilles ayant une saison de croissance plus longue aux faibles températures, les quantités de sucres que les plantes peuvent mettre en réserves peuvent être plus élevées si le taux de photosynthèse n'est pas affecté négativement par les baisses de températures, et que cela se traduit alors par une biomasse finale plus élevée.

Lapointe (2001) a alors récemment proposé une autre hypothèse pour expliquer l'effet de la température sur la croissance des éphémères de printemps. L'induction de la sénescence des parties aériennes pendant la période de croissance pourrait être influencée par la demande en C des organes de réserves (puits très important d'un point de vue quantitatif) plutôt que par des facteurs abiotiques. Au début de la saison de croissance, les plantes refont leurs réserves en sucres pour assurer leur croissance et la reproduction (Figure 1.2). La demande en C et donc la force des puits est très forte. Le bulbe représente le plus fort puits étant donné que c'est lui qui permet le stockage des ressources (sucres et nutriments), même si d'autres puits, liés à la croissance de l'organe pérenne, des fruits (pour certaines

espèces) et des feuilles, ainsi qu'à la respiration cellulaire, sont également actifs pendant la croissance épigée. Le modèle explique le maintien des parties aériennes par la présence de cette demande en ressources de la part des puits. La sénescence irréversible des feuilles serait induite lorsque cette demande n'existerait plus, et tout particulièrement lorsque le bulbe serait saturé en réserves. Dans le cas des températures élevées, la longévité plus faible des feuilles pourrait s'expliquer par une décroissance plus rapide, au fil de la saison de croissance, de la demande totale des puits. La saturation plus rapide de la demande des puits aux températures élevées pourrait être reliée au taux de croissance de l'organe pérenne, traduisant une modification de la capacité d'accumulation des sucres suivant son état de développement (taux de division cellulaire et élongation). D'après ce modèle, la température aurait un effet non pas direct sur la longévité des feuilles, mais indirect via son impact sur la croissance de l'organe pérenne.

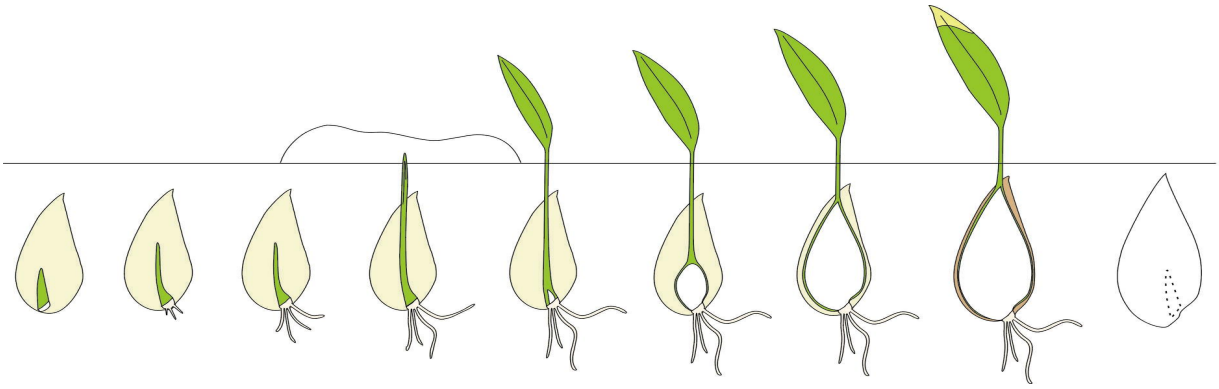

Figure 1.2. Illustration représentant le stade de développement d'un bulbe en fin de période de dormance estivale, le développement du nouveau système racinaire au cours de l'automne, la croissance de la nouvelle feuille sous le manteau neigeux au cours de l'hiver, et enfin la formation du nouveau bulbe (en blanc) lors de la période de croissance épigée le printemps suivant chez les individus immatures d'érythrone d'Amérique qui ne produisent ni rhizomes, ni bulbes filles, ce qui était le cas dans la présente étude.

Problématique

La présente étude a pour but de mieux comprendre les facteurs qui influencent le taux de croissance et la longévité des feuilles chez les éphémères printanières pérennes. L'approche a consisté à moduler les forces des puits (bulbe) et des sources (feuille) de carbone de notre modèle d'étude, l'érythrone d'Amérique (*Erythronium americanum*), afin d'étudier les effets sur la croissance et la phénologie de la plante durant la période de croissance épigée.

La première expérience, qui est développée dans le chapitre 2, avait pour but de caractériser plus précisément les effets de la température sur la croissance et la physiologie de l'érythrone d'Amérique. Il a été montré qu'à un traitement de température plus élevé que celui observé normalement dans la nature au début du printemps, la longévité des parties aériennes de l'érythrone du Japon (Yoshie et Fukuda, 1994) et de celles de l'érythrone d'Amérique (Lapointe et Lerat, 2006) est réduite. Mais l'étude de Lapointe et Lerat (2006) a également montré que la taille finale du bulbe, ainsi que sa concentration finale en amidon, sont favorisées aux faibles régimes de température. Ces résultats contrastent donc avec les données généralement observées dans la littérature. En effet, l'augmentation de température est reconnue entraîner une augmentation des taux métaboliques de processus tels que la photosynthèse, la respiration et la translocation de carbone. Dans le cas contraire, lors de faibles températures, la quantité d'énergie disponible pour le maintien et la croissance des plantes est souvent insuffisante. L'organisation cellulaire est perturbée ce qui provoque un ralentissement du métabolisme, de la croissance des tissus et des organes via une diminution du taux de division cellulaire (Francis et Barlow, 1988) et une diminution du taux de photosynthèse. Car même si les plantes vivant en milieux froids sont très bien adaptées à ces températures, que ce soit d'un point de vue morphologique ou physiologique (Goryshina, 1972 ; Mamushina & Zubkova, 1996), il n'en reste pas moins qu'à ce jour très peu d'espèces ont montré une plus forte croissance des plantes à des températures froides à l'exception de quelques plantes arctiques (Heide, 1992 ; Heide & Gauslaa, 1999). Des bulbes d'érythrone récoltés en milieu naturel ont été exposés à deux traitements de température, un « frais » et un « plus chaud », en chambre de croissance. Par

la suite, des mesures de croissance de la plante entière et des différents organes dans le temps, de concentration et de contenu d'amidon dans le bulbe, et enfin de taille et de nombre de cellules dans le bulbe après sénescence de la feuille ont été réalisées. Si la sénescence des feuilles est induite par une baisse de l'activité des puits, on s'attend à ce que la masse des bulbes cesse de croître avant que n'apparaissent les premiers symptômes de sénescence des feuilles. Nous voulons aussi caractériser l'influence de la température sur la croissance du bulbe en déterminant lequel du nombre ou de la taille des cellules a un effet prépondérant sur la taille finale du bulbe.

Dans un deuxième temps (chapitre 3), nous voulions savoir s'il était possible d'induire une sénescence précoce de la feuille. Pour cela, des bulbes d'érythrone ont été placés dans des conditions enrichies en CO_2 afin d'augmenter l'activité photosynthétique des feuilles (source), et des bulbes identiques ont été placé dans des conditions ambiantes de CO_2 afin de servir de témoins. De nombreuses études ont montré le bienfait d'une augmentation de la concentration en CO_2 sur la croissance des plantes, en particulier pour les plantes en C_3 (Bowes, 1991 ; Farrar & Williams, 1991 ; Lawlor & Mitchell, 1991 ; Grodzinski, 1992). L'augmentation de CO_2 provoque une augmentation de l'assimilation du C par les feuilles, de l'accumulation de sucres (en particulier d'amidon) et du taux de photosynthèse nette. Cette augmentation est liée à la fois à une diminution de la photorespiration provoquée par la diminution du ratio O_2/CO_2 ainsi qu'à l'effet direct de l'augmentation du CO_2 sur l'activité de la Rubisco. En fournissant plus de CO_2 à la plante, nous espérions augmenter la vitesse de remplissage du bulbe et donc accélérer le processus de sénescence des parties aériennes. Mais en exposant les plantes à des températures semblables dans les chambres, une biomasse finale semblable aux deux températures était attendue.

Chapitre 2 - Temperature effects on bulb growth and leaf longevity of the woodland spring ephemeral, *Erythronium americanum*

2.1. AVANT-PROPOS

Ce chapitre a été présenté sous forme d'affiche au XXII Congress of the Scandinavian Plant Physiology Society qui a lieu à Umeå (Suède) en juin 2005 et une partie a fait l'objet d'une présentation orale au 2ème colloque conjoint entre le CRBF (Centre de Recherche en Biologie Forestière) et le GREFi (Groupe de Recherche en Écologie Forestière interuniversitaire) qui s'est tenu en mars 2004 à l'Université Laval, Québec. Rachel Gauci a participé à la cueillette des données durant la phase expérimentale de ce travail.

2.2. RÉSUMÉ

Les plantes éphémères printanières des forêts décidues sont caractérisées par une courte saison de croissance épigée. Les parties aériennes apparaissent au début du printemps et quelques semaines plus tard, au moment où les feuilles des arbres se déploient, elles commencent à entrer en sénescence. Ici, nous avons testé l'hypothèse de l'induction de la sénescence de la feuille par une réduction de la demande en sucres de la part des puits. Nous avons cultivé des bulbes d'érythrone d'Amérique à deux régimes de température : 12/8°C et 18/14°C (jour/nuit). La phénologie de la feuille, la masse de la plante, la concentration en nutriments dans les feuilles, et le contenu en amidon, la taille et le nombre de cellules du bulbe ont été quantifiés durant la saison de croissance. Le taux de croissance initial de la plante était similaire aux deux régimes de température, mais les plantes cultivées à 18/14°C débutèrent à sénescer environ deux semaines plus tôt que celles cultivées à 12/8°C. Il en résulta une biomasse du bulbe significativement plus faible à la fin de la période de croissance épigée au régime de température plus élevé. La biomasse plus

élevée des bulbes à 12/8°C résultait davantage d'une élongation accrue des cellules que d'un nombre plus élevé de cellules. La température n'affecta pas le moment de la translocation des nutriments des feuilles. La concentration en amidon dans le bulbe n'était que sensiblement plus faible à 18/14°C qu'à 12/8°C et a atteint des valeurs maximum très tôt dans la saison. Il semblerait donc que le stockage d'amidon ait lieu au fur et à mesure de la croissance des cellules du bulbe, et que plus la saison de croissance est longue, plus les plantes accumulent de biomasse. Le fait que le bulbe arrête de croître alors que la quantité de nutriments dans les feuilles est encore élevée supporte l'hypothèse que la croissance de la plante et la durée des feuilles chez les éphémères printanières seraient déterminées par le potentiel de croissance des puits.

2.3. ABSTRACT

Deciduous forest spring ephemerals are characterized by a short epigeous growth season. Shoots appear in early spring, and in a matter of weeks, once tree leaf canopy is expanding, they start to senesce. Here, we tested the hypothesis that leaf senescence is induced by a reduction of carbohydrate sink demand. We cultivated *Erythronium americanum* bulbs under two day/night temperature regimes: 12/8°C and 18/14°C. Leaf phenology, plant biomass, leaf nutrient concentration, starch content and cell size and number in the bulb during the growing season were quantified. Initial plant growth rate was similar at both temperatures but plants grown at 18/14°C started to senesce about two weeks before those grown at 12/8°C. This leads to a significantly lower final bulb biomass at the higher temperature regime. The greater bulb biomass at 12/8°C resulted from greater cell size rather than from a greater number of cells. Temperature did not affect the timing of nutrient remobilisation from the leaves. Bulb starch concentration was only slightly lower at 18/14°C than at 12/8°C and reached maximum values very early in the season. It thus seems that starch storage was able to keep up with bulb cell elongation rate and that the longer the growth period, the more biomass plants accumulated. The fact that bulb stopped to grow while leaf nutrient levels were still high supports the hypothesis that sink growth potential determines plant growth and leaf lifespan of spring ephemerals.

2.4. INTRODUCTION

Literature on the mechanisms of plant senescence and especially of leaf senescence is abundant (Thomas & Stoddart, 1980; Noodén, 1988; Buchanan-Wollaston *et al.*, 2003 and references therein). Senescence is known to be an active process, allowing plants to recycle their nutrients and is controlled by internal and external factors. Changes that occur in plants during senescence, such as nutrient reallocation, chloroplast breakdown, cell disassembly and gene activation or repression are well known. However the determinism of senescence, i.e. the understanding of the nature and origin of the signal(s), is complex to establish, particularly in herbaceous plants. Under optimal conditions, leaf senescence of perennial plants appears to be mainly influenced by photoperiod, as observed in temperate trees and shrubs. Internal factors such as sink strength have also been reported as potential triggering signal for leaf senescence (Feller & Fisher, 1994; Egli, 2004). For example, sugars that are known to play an important role in regulation of plant metabolism (Smeekens, 2000; Rolland, More & Sheen, 2002) are more and more considered as potential actors in senescence signalling.

Spring ephemerals are commonly-found herbs in forests of North America that are characterized by a short leaf lifespan. These plants are believed to have evolved to take advantage of the temporary conditions of high luminosity and nutrient availability, and the weak inter-specific competition preceding canopy closure and the development of summer herbaceous plants in the understorey (Sparling, 1964; Muller, 1978). Shoots appear during, or a little time after snowmelt and in a matter of weeks the plant completes its epigeous growth cycle. Forty to sixty days of growth later, aboveground parts senesce (Lapointe, 2001). During this period, plants store a large fraction of the photoassimilates in their belowground organ (rhizome, corm, or bulb) for the next growing season. Because in nature shoot yellowing of spring geophyte ephemerals occurs when tree leaf canopy is expanding, the life cycle of these species was initially associated with sunlight intensity at the ground level (Sparling 1964, 1967; Vézina & Grandtner, 1965; Taylor & Pearcy, 1976).

These observations were consistent with the findings that ephemeral growth may be highly affected by a shading treatment (Eickmeier & Schussler, 1993).

However, although changes in light in the understorey seem appealing as a trigger for leaf senescence in spring ephemerals, they also senesce after a few weeks when maintained under constant light conditions. Thus, other studies focused on temperature, which was suspected to be responsible for yearly variations in growth and phenology. Length and intensity of the cold period were shown to impact on the emergence, the longevity and the size of *Claytonia virginica*, *Erythronium americanum* and *E. albidum* (Risser & Cottam, 1967). A correlation between leaf development and air temperature summation was also noticed in *E. americanum* (Muller, 1978) and an increase in growth temperature was shown to decrease leaf longevity in *E. japonicum* (Yoshie & Fukuda, 1994). But since bulb growth was also favoured by low temperatures in many spring ephemerals (Le Nard and De Hertogh 1993; Nault and Gagnon 1993) a new hypothesis was proposed linking leaf senescence to a reduction of carbohydrate sink demand (Lapointe, 2001). Positive impact of low temperatures on both leaf life duration and bulb growth has now been reported in *E. americanum* (Lapointe & Lerat, 2006) and in *Crocus vernus* (Badri *et al.*, in preparation). We believe that leaf lifespan is modulated by the storage capacity of the plant. In other words, the bigger the bulb becomes, the longer the leaf stays green. The irreversible senescence of the leaf would occur when C demand falls, thus, when the bulb is full or when its growth has strongly reduced or ceased. In such case, environmental factors would have an indirect effect on leaf senescence via their impact on the growth of belowground parts (cell division or elongation) or the rate of C storage rather than a direct effect on the leaf senescence process.

In an attempt to test this hypothesis, we grew *E. americanum* plants under two day/night temperature regimes: 12/8°C and 18/14°C. We studied leaf phenology and quantified the growth of the above- and belowground organs during the whole epigeous growth season. Starch accumulation and final cell size and number in bulbs were also investigated as measures of sink strength. To better characterize the onset of leaf senescence, examination

of nutrient mobilization from the leaf completed the visual observations. The aim of this work was to better understand the factors controlling source-sink relations in this spring ephemeral. Particularly, we wanted to know whether bulb size at the end of the epigeous growth season is controlled more by a temperature effect on leaf ageing (Yoshie & Fukuda, 1994) or by a direct temperature effect on bulb growth processes.

2.5. MATERIALS AND METHODS

2.5.1. Plant material and growing conditions

Bulbs of non-flowering *E. americanum* plants were harvested late April 2003, after snowmelt, in a sugar maple forest belonging to Université Laval at Saint-Augustin-de-Desmaures near Québec City (46°48' N, 71°23' W). Bulbs were sorted by size, weighed (only bulbs between 0.3 and 0.5 g were retained) and planted in 10 cm plastic pots filled with Turface (Applied Industrial Materials, Corp., Buffalo Grove, Ill., USA). Pots were placed in growth chambers (PGW36, Conviron Inc., Winnipeg MB, Canada). Two growth conditions were used, and each one was applied to eighty one plants. The first supplied a 12/8°C (day/night) temperature (Cool Temperature Treatment, CT), a photosynthetically active radiation (PAR) of approximately 300 μmol m^{-2} s^{-1} with 14-h d^{-1} photoperiod and 50% relative humidity (RH). The second supplied the same conditions for the light and the photoperiod but the temperature was set at 18/14°C (Warm Temperature Treatment, WT) with 75% RH. The two temperature regimes correspond to the mean day and night air temperatures recorded at the Quebec City airport weather station in the first two weeks of May (CT plants) and June (WT plants). RH in the two chambers was adjusted to submit the plants to similar water vapour pressure deficits despite differences in temperatures. Plants were watered daily and fertilized weekly with 150 mL of a 10% Hoagland's solution (Hoagland & Arnon, 1950) as this concentration has been shown to be optimal for *E. americanum* growth (Lapointe & Lerat, 2006).

2.5.2. Plant growth measurements

Seven plants per harvest and per temperature were sampled for a total of six harvests: when plants were harvested in the field (Harvest 1), during leaf growth (Harvests 2 and 3), at the beginning of leaf yellowing (Harvest 4), when half the leaf was yellowed (Harvest 5) and the last one once the leaf had completely senesced (Harvest 6). Leaf area (Harvest 2 to 4) and fresh and dry weights (6 harvests; freeze-drying for 48 h) of the leaf, the bulb and the roots were determined for each plant. Two phenological variables were followed more precisely during the experiment: the duration of the mature phase and leaf longevity. The length of the mature stage was determined as the mean period between complete leaf unfolding and appearance of the first symptoms of senescence on the leaf (yellowing of the tip). Leaf longevity was determined as the mean period between leaf unfolding and its complete senescence, i.e. once the chlorophyll is completely degraded and the leaf falls on the ground. In other studies (Yoshie & Fukuda, 1994; Lapointe & Lerat, 2006), the duration of the period of emergence was included in these two phenological variables. We did not measure the length of this stage because leaf began to unroll before they were moved to their respective growth chambers. Once harvested in the field, all plants were potted and kept 3 days under the same conditions (12/8°C) to allow them to recover from the stress of transplantation. Then, plants were randomly placed in the growth chambers, but they had already started to unfold their leaf at that time.

2.5.3. Nutrient analysis

After dry weight was measured, leaves were ground (N = 7) and sent for N, P, and K analysis to the Laboratoire Daishowa, Pavillon de l'Envirotron, Université Laval. The N and P concentrations were determined colorimetrically after digestion with sulphuric and selenic acids and hydrogen peroxide. Concentration in K was determined by atomic absorption spectroscopy. Leaf nutrient content was estimated from nutrient concentration and leaf biomass.

2.5.4. Starch quantification

Starch concentration was measured in the bulbs since it is the main source of carbohydrate for storage in *E. americanum* (Risser & Cottam, 1968; Lapointe & Molard, 1997), representing 80-90% of the total bulb biomass when grown in control environments. Seven plants from Harvest 2 up to complete leaf senescence were sampled per harvest and per treatment. Bulb starch content was assayed according to the colorimetric method of Blakeney & Mutton (1980) as modified by Castonguay, Nadeau & Simard (1993). Fifty mg of dried bulbs (70°C) were heated at 65°C for 20 min in a solution of methanol, chloroform and water (vol:vol:vol /12:5:3), ground with a Polytron (Kinematica, Switzerland) and centrifuged at 4°C. The non-soluble fraction in the pellet containing starch was then gelatinized in boiling distilled water for 90 min and then hydrolyzed at 55°C for 1-h using *Rhizopus* amyloglucosidase (Sigma Chemical Co., St-Louis, MO, USA). Finally, reducing sugars obtained by hydrolysis of the starch were quantified colorimetrically at 415 nm using a solution of *p*-hydroxybenzoic acid hydrazide (PAHBAH) and starch concentration was determined by comparison with a standard curve.

2.5.5. Determination of cell size and number

Four bulbs per treatment from the final harvest were fixed in a FAA (Formaldehyde - Acetic Acid – Alcohol) solution (Sass, 1958) and embedded in paraffin. Then, transverse sections were taken in the middle of the bulb. The sections were mounted on standard microscope slides, stained with 0.01% (w/v) toluidine blue and observed under light microscope (Olympus, USA). Digital pictures were taken with a camera fixed on the microscope. Cell area and number were measured with the 3D-Doctor software (Able Software Corp., Lexington MA, USA).

2.5.6. Experimental design and statistical analysis

The experiment was conducted in controlled environment growth chambers. Two chambers were used, one for each treatment. Although plants inside were not true replicates (Hurlbert, 1984) they were considered as such in this study. To be sure our observations were the result of treatment effects and not those of a potential growth chamber effect or a year effect (Potvin & Tardif, 1988), two complementary experiments were conducted, one where growth chambers were switched between treatments (in 2005) and one that was conducted by another investigator (in 2006). Only the results of 2003 are shown in the present study.

Two-way ANOVAs with time and treatment as main factors, followed by a *posteriori* multiple comparisons using Fisher's LSD tests, were performed to compare bulb starch content, bulb starch concentration, bulb biomass, foliar nutrient content and foliar nutrient concentration. T-tests were performed to compare cell size and cell number at the end of the epigeous growth period, duration of the mature phase, leaf longevity, as well as mature leaf size and leaf biomass (Harvest 4). All statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA). A critical value of $\alpha=0.05$ was used for the tests of significance, and data were transformed when the assumptions of homogeneity of variance and normality were not met.

2.6. RESULTS

2.6.1. Plant growth

Temperature impacted *E. americanum* growth, particularly bulb growth (Figures 2.1 and 2.2b). However, the kinetics of plant response differed between the two temperature regimes because the time × treatment interaction was significant ($F_{5,65}=8.08$; $P<0.001$). During the first ten days, bulb growth rate was greater at 12/8°C than at 18/14°C. Two

weeks after leaf unfolding, WT plants caught up with the CT plants and reached similar bulb biomass. One week later, CT and WT plants continue to present similar biomass. The average bulb dry mass (DM) was then 97 ± 7 mg for CT plants (day 20) and 94 ± 13 mg for WT plants (day 23). However, at that time WT plants had begun to senesce whereas CT plants continued to grow for two more weeks (similar observations were done in 2005; Figure 2.2a). At the end of the epigeous growth season, bulbs of CT plants were 2.8 times heavier than those of WT plants. Average bulb DM were 177 ± 13 mg for CT plants and 64 ± 4 mg for WT plants, which represented a biomass gain of about 360 % and 70%, respectively. Microscopic measurements done on the bulbs harvested after complete leaf senescence showed that cells were larger at 12/8°C than at 18/14°C (t=5.6, *P*=0.001; Fig. 2.3). The cell counts revealed no significant difference in average cell number although CT bulbs tended to show slightly higher numbers than WT bulbs (t=2.4, *P*=0.053).

Leaf mass at the onset of yellowing was not affected by temperature (*t*=2.04; *P*=0.067), with average value of 32 ± 2 mg for CT plants and 26 ± 3 mg for WT plants (Figure 2.4). Nevertheless, CT plants produced larger leaves. The leaf size of CT and WT plants were not different when senescence occurred in WT plant (*t*=1.43; *P*=0.189), but the final leaf size of CT plants was nearly 40% larger than that of WT plants (*t*=4.13; *P*=0.002). Average values of leaf area were 7.2 ± 0.4 and 5.1 ± 0.4 cm^2 for CT and WT plants, respectively.

2.6.2. Leaf longevity

Plant growth phase duration (t=5.7; *P*<0.001) and leaf longevity (t=14.0; *P*<0.001) were affected by temperature. For both variables, average values were higher at 12/8°C than at 18/14°C (Figure 2.1). First senescence symptoms of CT plants appeared in a synchronous way about 34 days after complete leaf unfolding. The leaf died on average 12 days later, for a total leaf longevity of 46 days. In the case of WT plants, the leaf began to senesce on average 23 days after leaf unfolding, i.e. 11 days before CT plants. But the first symptoms were not synchronous. As soon as 18^{th} day, some leaves were beginning to turn yellow. The average leaf longevity was 32 days, i.e. 2 weeks shorter than for CT plants.

2.6.3. Bulb starch storage

Bulb starch content increased with time, but the duration of starch accumulation differed greatly between the two temperature regimes (interaction time × treatment: $F_{4,54}$=45.43; P<0.001; Figure 2.5a). In WT plants, starch accumulated in the bulb until the onset of leaf yellowing (23d), and starch content at complete leaf senescence was significantly lower than at the onset of yellowing. In CT plants, starch content still increased after the beginning of leaf senescence (up to 40d). A decrease in starch content between half-yellowing and complete leaf senescence was observed, but the final starch content was not significantly different from that measured at the onset of yellowing. Final starch content was 3 times higher for CT plants (167 ± 4 mg) than for WT plants (53 ± 1 mg).

No significant interaction was found between time and treatment on bulb starch concentration (Figure 2.5b). However, a significant effect was found for time ($F_{4,54}$=46.85; P<0.001) and temperature ($F_{1,54}$=32.12; P<0.0001), indicating that the shape of the response curves was similar for both treatments. Starch concentrations increased during the first 20 days of growth at 12/8°C and during the first 14 days at 18/14°C. No significant differences were found for concentrations between harvests 3 and 6. Bulb starch concentration was slightly higher in CT plants than in WT plants. At complete leaf senescence, respectively 898 ± 51 and 838 ± 19 mg of starch g^{-1} bulb dry matter were measured in CT and WT plants.

2.6.4. Foliar nutrient status

N and P foliar concentrations decreased with time (N, $F_{4,54}$=44.2, P<0.001; P, $F_{4,43}$=13.7, P<0.001) and were significantly greater at each harvest in WT than in CT plants (N, $F_{1,54}$=20.3, P<0.001; P, $F_{1,43}$=6.1, P=0.02; Figure 2.6). However, according to the shape of the curves, daily changes in P and N concentrations were very similar at both temperatures. No treatment effect was noticed for K (K, $F_{1,48}$=3.3, P=0.07), but its concentration changed

with time (K, $F_{4,48}$=33.4, P<0.001). K concentration dropped by 80% between the 2nd and the final harvest.

No significant interactions between time and treatment were found on N, P and K foliar contents. The content in these nutrients was greater in CT than in WT plant leaf (N, $F_{1,54}$=7.5, P<0.01; P, $F_{1,43}$=6.9, P=0.01; K, $F_{1,48}$=4.3, P=0.04; Figure 2.6) and they fluctuated with time (N, $F_{4,54}$=93.3; P, $F_{4,43}$=14.1; K, $F_{4,48}$=18.1, P<0.001). P and K contents were stable until the onset of leaf yellowing and decreased afterwards. N content began to decrease from the third harvest (Day 14 and Day 20 at 18/14°C and 12/8°C respectively).

2.7. DISCUSSION

Temperature effect on whole plant growth

Our results demonstrate that the regime of temperature had an impact on overall *E. americanum* growth. Total plant biomass at the onset of leaf yellowing, and at the end of the epigeous growth period was lower in WT plants compared to CT ones. But this difference mainly reflected a reduction in bulb growth (Fig. 2.1), because neither root (data not shown), nor leaf mass (Fig. 2.4) was affected by the treatments. However, such a low optimum temperature for growth is quite unusual, even amongst plants living in extreme climates. Studies on alpine and arctic herbs generally report positive or null effects of a temperature increase on vegetative growth and reproductive output, depending on the species geographic location (high/low arctic or alpine environments) and functional groups (Chapin *et al.*, 1995; Henry & Molau, 1997; Molau, 1997; Arft *et al*., 1999). At least, no significantly-lower vegetative growth is reported. Greater biomass accumulation and reproductive ratio at higher temperatures were even reported in *Floerkea proserpinacoides*, a spring ephemeral annual living in deciduous forests of North America (McKenna & Houle, 2000). Examples that support our findings are not numerous in the literature. A better growth at temperatures around 9-12°C was found in the high-alpine grass *Phippsia*

algida (Heide, 1992), and the high-arctic annual *Koenigia islandica* (Heide & Gauslaa, 1999) and certain cultivars of onion also exhibit better growth at decreasing temperatures between 12°C and 18°C (Daymond *et al.*, 1997). Lower temperatures have also been found to impact positively the reproductive success of *Ranunculus glacialis*, compared to other related arctic/alpine species (Totland & Alatalo, 2002). Although there has been many global warming studies conducted in the field in the last ten years, the results are not easily brought back to the scale of the individual. By contrast to experiments done under controlled environments, temperature manipulations in the field often result in numerous interactions between abiotic variables, such as changes in soil temperature and moisture, and nutrient availability (Chapin *et al.*, 1995) that makes temperature effects difficult to isolate. Therefore, to this point, deciduous forest spring ephemerals appears quite unique in their response to growth temperature.

Temperature effects on leaf growth

When WT plants started to senesce, leaf size and biomass of CT and WT plants were not significantly different (Fig. 2.4). Thus, we believe the larger leaves of CT plants at leaf yellowing were the consequence of their longer leaf lifespan; it has been shown that spring ephemeral leaves grow throughout the epigeous growth period (Zubkova *et al.*, 1997). Nevertheless, we cannot exclude the possibility that an imbalance between water influx and efflux, triggered by a greater evaporative demand in WT plants, might have (caused a potential inhibition of cell growth (Boyer, 1985), without a decrease in turgor pressure (Boyer, 1988), despite daily plant watering. Spring ephemerals are characterized by high stomatal conductances and transpiration rates (Taylor & Pearcy, 1976), even when climatic conditions are not favourable (Goryshina, 1972). Indeed, WT plants exhibited higher transpiration rates than CT plants (A. Gandin, unpubl. data). However, since leaf sizes were similar between the two temperatures up to the point where WT plants started to senesce, it is most probable that larger final leaf size of CT plants reflects longer leaf life duration rather than an imbalance in water fluxes at the higher temperature regime.

Temperature effects on bulb growth and starch storage

Biomass gains of 360 and 70% were found after complete shoot senescence at low and high temperature regime, respectively, for the bulb. However, this final biomass and size disparity was not representative of what happened during the complete epigeous growth cycle. During the first twenty days of growth, biomass accumulation in the bulbs of CT and WT plants was very similar. This suggests that temperature had little impact on bulb growth rate and agree with the findings that photosynthetic rates are only slightly enhanced by the higher temperature regime (A. Gandin, unpubl. data). Biomass changes became visible when the leaf of WT plants started to yellow, whereas CT plants continued to grow. At that time, CT plants had only reached 45% of their final biomass. Maximal starch concentration was also reached very quickly (Harvest 3; Fig. 2.5b). Given this result and that the final change in bulb size was accounted mainly by a change in cell enlargement rather than in cell number, one can expect that subsequent biomass gains were related to bulb cell enlargement and their gradual filling with starch. Even if starch concentration was slightly higher in CT plants than in WT ones, this difference (respectively 898 and 838 mg g^{-1} dry matter in CT and WT plants) can only explain a small fraction of the 2.8-time increase in bulb biomass of the plants grown at the lower temperature regime. In onion (Wheeler *et al.*, 2004) and crocus (Badri *et al.*, in prep.), two species that showed a reduction in bulb/corm size at higher temperatures, carbohydrate concentrations were not either responsible for changes in biomass. Furthermore, crocus also showed an increase in corm cell size at lower temperature regimes (Badri *et al.*, in prep.), suggesting that temperature directly affect the growth of the perennial organ of spring ephemerals.

Is leaf longevity related to leaf ageing or bulb filling?

E. americanum leaf response to air temperature was similar to that reported in the studies of Yoshie & Fukuda (1994) and Lapointe & Lerat (2006). Plants cultivated at the higher temperature regime started to senesce earlier (23d) compared to control plants (34d) that leaded to a two-week shorter leaf lifespan (Fig. 2.1). The question still remaining is

whether bulb growth and starch storage stopped when degradation of photosynthesis apparatus occurs, or when bulb cells stopped to enlarge, giving the leaf the signal(s) to senesce.

Yoshie & Fukuda (1994) attributed the shortened leaf longevity of spring ephemerals to an increased aging rate of shoots at elevated temperature because phenological-stage monitoring revealed shorter leaf extension, elongation and maturation durations at higher temperatures. But leaf senescence is characterized by numerous biochemical changes some of which appear well before the first symptoms are visible, such as nutrient mobilization and photosynthesis decline and are part of the leaf ageing process (Yoshida, 2003). In WT plants, nutrient mobilisation only started at the onset of leaf yellowing, as shown by N, P and K content values (Fig. 2.6). This was different in CT plants, where nutrient content started to decrease earlier than in WT plants and ahead of the first visual sign of senescence. Nutrient concentration at leaf yellowing was greater in WT plants than in CT plants, particularly for nitrogen (Fig. 2.6), suggesting that the onset of leaf senescence was independent of nutrient concentration in this species. But most importantly, these results strongly suggest that leaves were still quite functional when bulb growth slowed down and leaves started to yellow. This hypothesis is supported by results reported elsewhere where significant assimilation rates were recorded throughout the leaf yellowing period allowing the plants to continue to accumulate carbohydrates (Gutjahr & Lapointe, *in preparation*).

In conclusion, these results do not support the hypothesis of an acceleration of the leaf ageing process at high temperature as put forward by Yoshie & Fukuda (1994). Changes in leaf nutrient content were not fastened at the higher temperature regime, and bulb growth ended at 18/14°C while leaf nutrient content was still high. Theses observations suggest that leaf senescence occurred prematurely in WT plants, and bring support to the hypothesis that leaf senescence is induced by sink limitation in *E. americanum*. The metabolic regulation of photosynthesis has been shown to involve high leaf sugar/nitrogen ratios (Wingler *et al.* 2006; Paul & Driscoll, 1997). This might be worth studying in an attempt to identify the signal that relates the induction of leaf senescence with the end of the bulb growth in *E. americanum*.

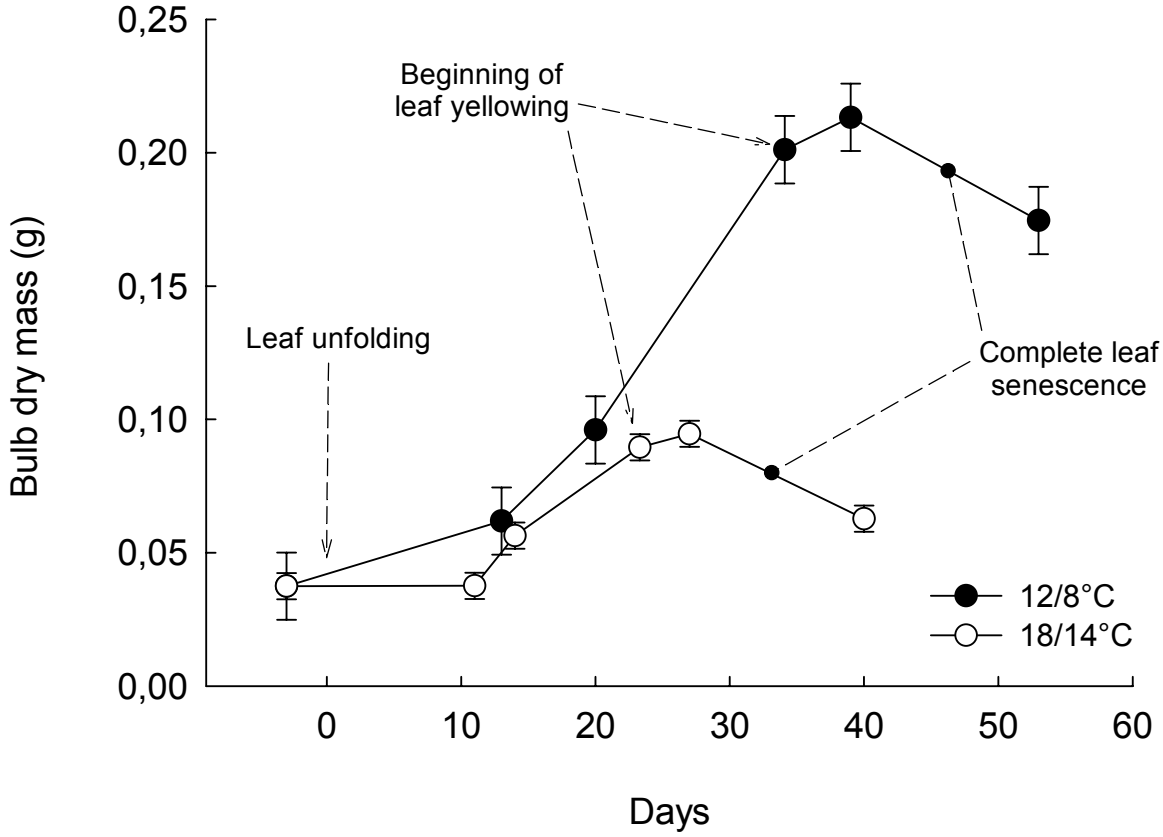

Figure 2.1. Bulb growth kinetics of *E. americanum* plants grown at 12/8°C (close symbols) and 18/14°C (open symbols) during the epigeous growth period. Data points represent means ± 1 SE from 7 plants. Harvest 1 corresponds to the time at which plants were harvested in the field. Leaf unfolding occurred simultaneously under both treatments (three days later), as explained in the method section and corresponds to the time of random distribution of potted-plants in each growth chamber. Harvests 2 and 3 took place during leaf growth, Harvest 4 when leaves began to yellow, and Harvest 5 when half the leaf had yellowed. Date of complete leaf senescence is shown by the dot for both treatments. The final harvest took place one week later (Harvest 6).

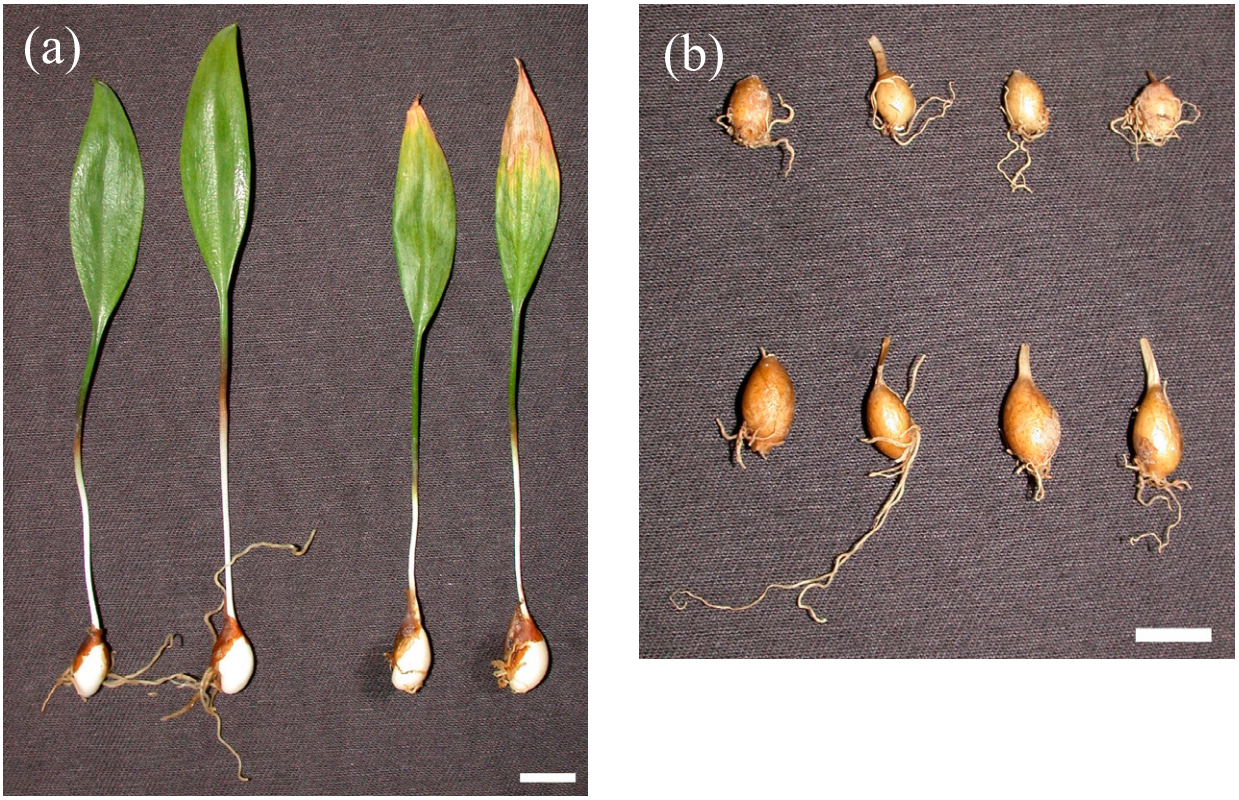

Figure 2.2. (a) Two representative *E. americanum* plants after 24 days of growth at 12/8°C (left) and 18/14°C (right) in 2005. The four plants had a similar size. Nevertheless, Warm Temperature (18/14°C) plants had started to senesce while Cool Temperature (12/8°C) plants continued to grow. (b) Bulbs after complete leaf senescence in 2003. The four bulbs above came from plants grown at 18/14°C. The four plants below were grown at 12/8°C. Bars represent 1 cm.

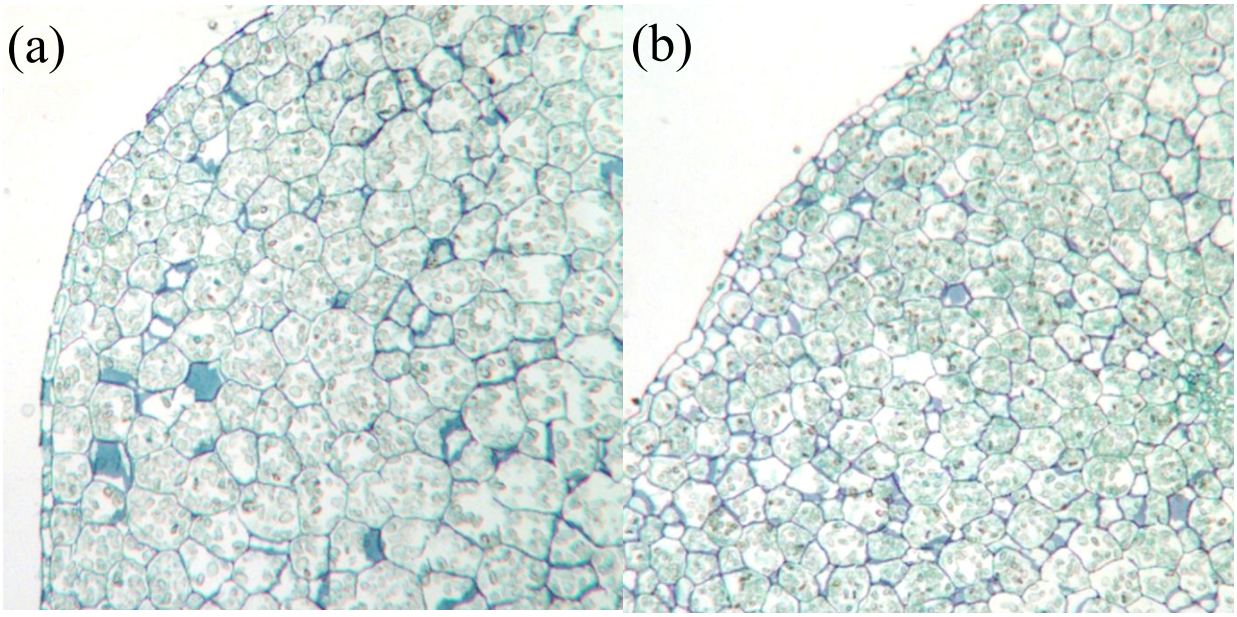

Figure 2.3. Digital pictures of two bulb transverse sections of *E. americanum* taken at the end of the epigeous growth period with a camera fixed on a light microscope (40×). Bulb (a) was grown at 12/8°C (Cool Temperature treatment) and (b) at 18/14°C (Warm Temperature treatment).

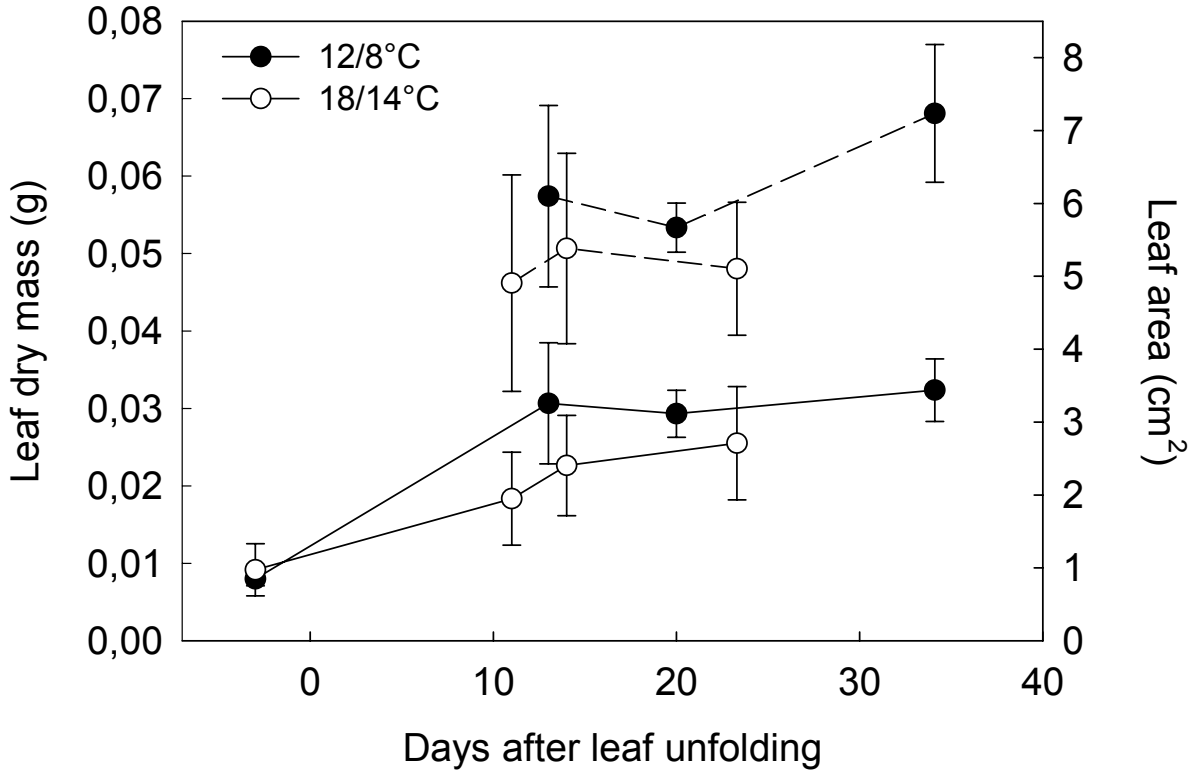

Figure 2.4. Variation of leaf characteristics of *E. americanum* plants from leaf unfolding to the onset of leaf yellowing (Harvest 1 to 4). Leaf dry mass (solid lines) and leaf area (dotted lines) of plants grown at 12/8°C (closed symbols) and 18/14°C (open symbols) are shown. Data points represent means ± 1 SE of 7 plants.

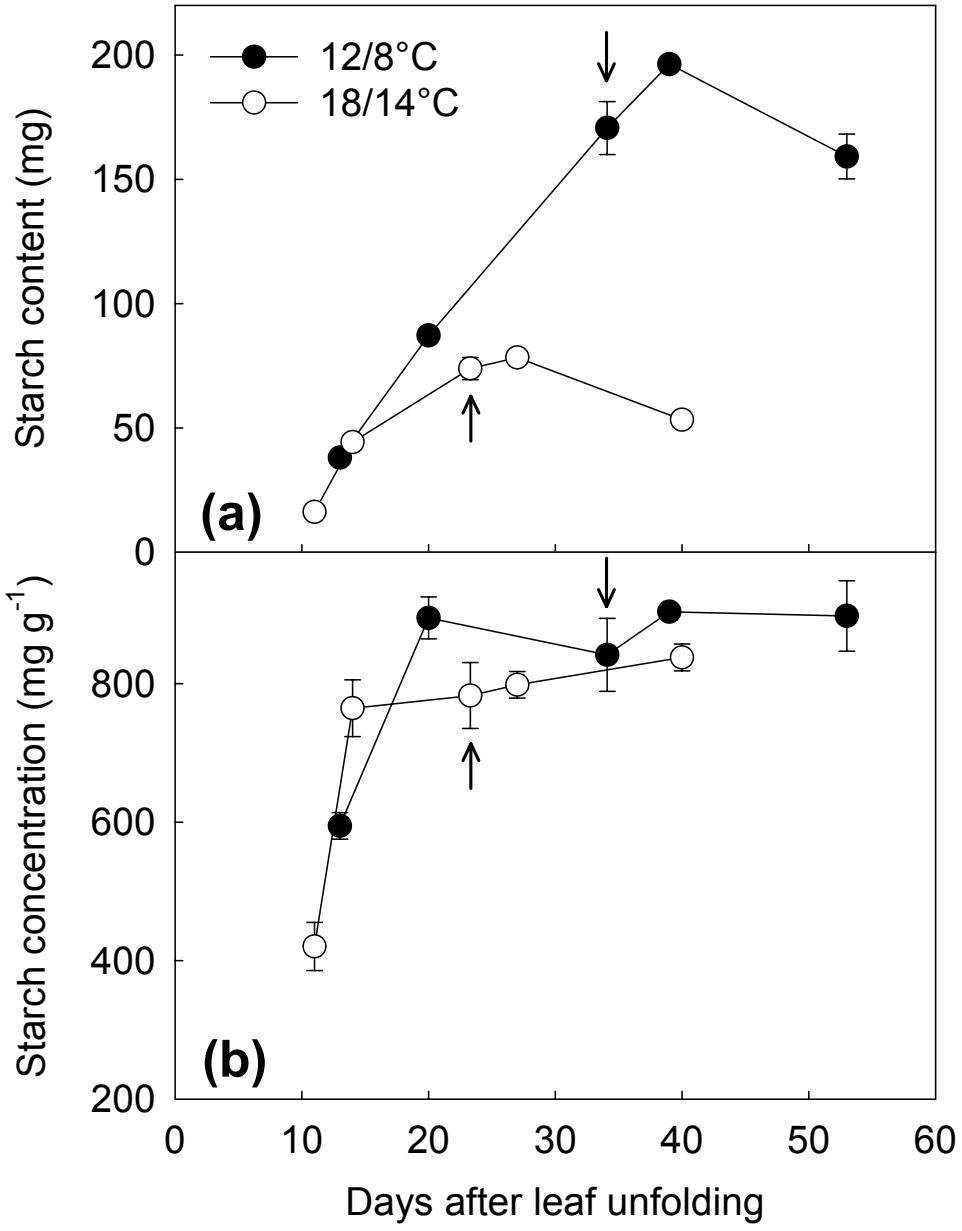

Figure 2.5. Variation of bulb starch content (a) and concentration (b) of *E. americanum* grown at 12/8°C (closed symbols) and 18/14°C (open symbols) during the epigeous growth period (Harvests 2 to 6). The arrows indicate the onset of leaf yellowing. Data points represent means ± 1 SE of 7 plants.

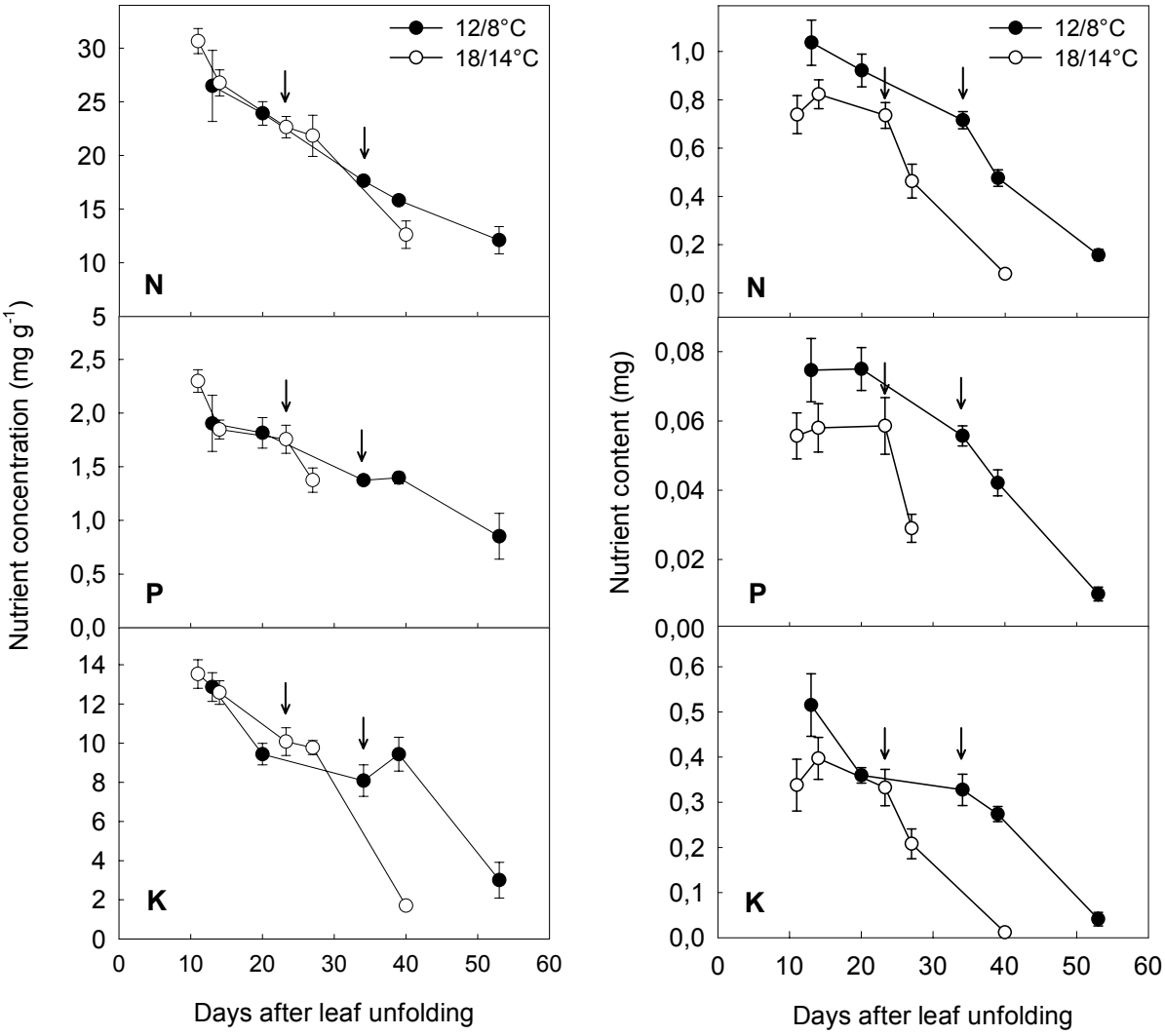

Figure 2.6. Foliar nutrient concentration (left) and content (right) of *E. americanum* plants grown at 12/8°C (closed circles) and 18/14°C (open circles) (Harvests 2 to 6). Means ± 1 SE are shown (n=3-7). The arrows indicate the onset of leaf yellowing. The final value of P concentration and content at 18/14°C could not been determined precisely by the equipment (values reported were inferior to 0.1 mg g^{-1}).

Chapitre 3 - Elevated carbon dioxide concentration influences neither bulb storage, nor leaf longevity of *Erythronium americanum*, a woodland spring ephemeral

3.1. AVANT-PROPOS

Une partie de ce chapitre a été présenté sous forme d'affiche au XXII Congress of the Scandinavian Plant Physiology Society qui a eu lieu à Umeå, en Suède, en juin 2005. Marie-Josée Drouin, Christophe Gouraud et Olivier Larouche ont participé à la phase expérimentale de ce travail.

3.2. RÉSUMÉ

Les plantes éphémères printanières présentent une période de croissance épigée relativement courte, qui a lieu entre la fonte de la neige et la fermeture du couvert forestier. La sénescence des feuilles était imputée à la diminution de luminosité en sous-bois, jusqu'à ce qu'il soit montré que la température a également un impact sur la longévité des feuilles et la croissance de la plante en général. Plus particulièrement, il a été montré chez certaines espèces géophytes que les faibles températures favorisent une biomasse finale plus importante de l'organe de réserve souterrain et une durée de vie plus longue des feuilles, un résultat qui amène à associer la sénescence des feuilles à la réduction de la demande en sucres des puits. Afin de tester cette hypothèse, des bulbes d'*Erythronium americanum* ont été cultivés à deux concentrations de CO_2 (élevée et ambiante) en chambres de croissance. Le but de cette étude était de déterminer si l'augmentation du taux de photosynthèse net des plantes en concentration de CO_2 élevée entraîne une réduction de la durée de vie des feuilles via l'accélération du remplissage du bulbe. La biomasse des plantes, la phénologie

et la concentration en nutriments de la feuille, et la quantité d'amidon dans le bulbe durant la période de croissance épigée ont été déterminées à deux reprises, en 2004 et 2005. Les plantes cultivées en conditions élevées en CO_2 avaient un taux d'assimilation net plus élevé que celles cultivées en milieu ambiant. Le traitement n'a eu aucune influence sur la taille des plantes et la biomasse finale du bulbe. Contrairement à nos prédictions, les plantes cultivées en concentration élevée en CO_2 n'ont pas bénéficié du surplus de C. Le remplissage du bulbe n'a pas été accéléré et les feuilles ne sont pas entrées en sénescence plus rapidement. Ce résultat serait possiblement causé par une augmentation du taux de respiration de bulbe et de la feuille. Le taux de croissance similaire du bulbe aux deux concentrations en CO_2 suggère que la vitesse de remplissage du bulbe est limitée par la vitesse d'élongation des cellules plutôt que par la disponibilité du C.

3.3. SUMMARY

Woodland spring ephemerals exhibit a relatively short epigeous growth period between snowmelt and canopy closure. Leaf senescence was considered to be due to changes in light in the understorey, until temperature was shown to impact on leaf longevity and plant growth. More particularly, lower temperatures were shown to favour larger final bulb mass and longer leaf lifespan, a finding that leaded to link leaf senescence to a reduction of carbohydrate sink demand. To test this hypothesis, *Erythronium americanum* bulbs were grown under two CO_2 concentrations (elevated and ambient) in controlled environments. The goal of this study was to investigate whether the increase in net assimilation rate of the plants under elevated CO_2 concentration would shorten leaf lifespan due to more rapidely-filled bulbs. Plant biomass, leaf phenology and nutrient concentration, and starch amount in the bulb during the growing season were determined twice, in 2004 and 2005. Plants grown under elevated CO_2 concentration had greater net assimilation rates than those grown at ambient CO_2 concentrations. The treatment did not impact on plant size and final bulb mass. Contrary to our expectations, plants grown under elevated CO_2 did not benefit from the surplus of C supply. Bulb filling did not accelerate and leaves did not senesce earlier. These results could potentially be explained by an increase in leaf and bulb respiration rates

under elevated CO_2. The similar bulb growth rate under both CO_2 concentrations suggests that bulb filling rate is dependant on bulb cell elongation rate rather than on C availability.

3.4. INTRODUCTION

Spring ephemerals are commonly-found herbs in deciduous forests of North America. Most of them are clonal and perennial (Whigham, 2004) and are characterized by a relatively short epigeous growth period between snowmelt and canopy closure (Lapointe, 2001). During the 40 to 60 days of aboveground activity, spring ephemerals grow and may reproduce. In the same time, the perennial organ is renewed and resources such as nutrients and carbohydrates are stored in it for the next year. Thereafter, plant shoots senesce while belowground parts fall in dormancy, waiting for environmental signals to start a new growing season.

Because spring ephemerals exhibit some characteristics of sun plants (Sparling, 1964, 1967; Taylor & Pearcy, 1976) and their shoots begin to senesce while canopy is closing (Vézina & Grandtner, 1965), light reduction was thought to be the main signal responsible for leaf senescence. However, several studies have shown that spring herbs are also sensitive to other endogenous and exogenous factors. But to date, little is known about the impacts of these factors on biomass allocation and source-sink relationships in spring ephemerals. The senescence of the woodland spring annual *Floerkea proserpinacoides* has been shown to be correlated more with reproduction than with changes in light conditions (Ben Mokhtar & Houle, 2005). Vézina and Grandtner (1965) suspected the temperature to impact on perennial spring herb development, a belief strengthened by Muller (1978) who noticed a correlation between leaf development and air temperature summation rather than canopy closure in *Erythronium americanum*. Risser and Cottam (1967) demonstrated that temperature during the cold period as well as its duration impacted on the timing of shoot emergence, shoot lifespan and final plant height, and Yoshie and Fukuda (1994) reported negative effects of warm air temperatures during shoot growth period on *Erythronium japonicum* phenology. Recently, Lapointe and Lerat (2006) have shown in *E. americanum*

that warmer temperatures slightly shorten leaf longevity while inducing a strong decrease of the final bulb mass. It is thus possible that leaf lifespan of these species is correlated with carbon (C) sink maintenance, and particularly with carbohydrate storage in the perennial organ.

Based on these results, the present study was conducted to understand whether signals inducing shoot senescence are initiated by leaf (source) component break down or by reduction of bulb sink strength once it has completed its growth and is filled with C. More precisely, we wanted to investigate the possibility of altering leaf senescence of a spring perennial by modulating source strength. Bulbs of *E. americanum*, a common spring ephemeral of North American forests, were cultivated in growth chambers under ambient and enriched carbon dioxide (CO_2) concentration. The experiment was performed twice, in 2004 and 2005. The effects of CO_2 concentration on bulb starch content and concentration, gas exchanges, and plant growth were investigated. A positive response on net photosynthesis rate involving a greater carbohydrate exportation to the bulb, without effect on its growth (for example on cell division and elongation) and thus without changes in sink demand was expected. Therefore, bulbs might be filled more rapidly and leaf senescence hastened. The validation of our hypothesis would mean that growth capacity of the belowground part has to be taken into account to predict the response of spring perennial herbs to changes in CO_2 concentrations, and thus to global warming.

3.5. MATERIALS AND METHODS

3.5.1. Experimental design

Bulbs of *E. americanum* were harvested in September 2003 and 2004 in a maple forest in Saint-Augustin-de-Desmaures near Québec City, Canada (46°48’ N, 71°23’ W). Bulbs between 0.30-0.35 g fresh mass were selected, planted in 10 cm plastic pots filled with Turface (Applied Industrial Materials, Corp., Buffalo Grove, Ill.) and kept in a cold

chamber (4°C). Five months later, plants were randomly transferred in two growth chambers (Conviron Inc., Winnipeg, Manitoba, Canada). To take into account a potential growth chamber effect (Potvin & Tardif, 1988), the growth conditions in the chambers were inverted between the two years. Both chambers supplied a day/night temperature of 18/14°C, a light of 290-310 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation) with a light/dark regime of 14/10 h and 75% relative humidity (RH). CO_2 concentrations in each chamber were approximately 400 μmol mol^{-1} (ambient) and 1100 μmol mol^{-1} (elevated). Plants were grown at 18/14°C because at this temperature regime the maximum growth of this species is not reached (Lapointe & Lerat, 2006); hence, a potential effect of elevated [CO_2] on cell elongation or division might be detected. Plants were watered daily and fertilized weekly with 150 mL of a 10% Hoagland's solution.

3.5.2. Growth measurements

Respectively seven and six plants were harvested in 2004 and 2005, at six different times: before introduction of the plants in the chambers, during the growth period (2 harvests), when the first symptoms of leaf senescence appeared, in the middle of the leaf senescence and at complete leaf senescence. Leaf area (up to leaf yellowing) was measured with a leaf area meter (model 3100 Li-Cor Inc., Lincoln, NE, USA) and fresh and dry mass (70°C for 48 h) of the leaf, the bulb and the roots were determined. The length of the growth stage was determined as the period between complete leaf unfolding and the onset of leaf yellowing. Leaf longevity was determined as the period between leaf unfolding and complete leaf senescence.

3.5.3. Leaf gas-exchange measurements

Gas exchange measurements were collected using a portable LCA-4 infrared gas analyser (ADC Bioscientific Ltd, Hoddesdon, UK). Net leaf assimilation rates (A) were measured on the single-leaf of 12 plants per treatment. Measurements were taken on the same plants until leaf senescence in 2004, and during the two weeks following leaf unfolding in 2005,

in the afternoon. Rates of respiration were measured twice during the epigeous growth period in 2005, respectively 12 and 13 (leaves), and 13 and 18 days (bulbs) following leaf unfolding, after darkening and right before these plants were harvested.

3.5.4. Determination of cell size and number

After fixation in a solution of FAA (Formaldehyde - Acetic Acid – Alcohol; Sass, 1958), five bulbs per treatment were sectioned longitudinally, then mounted on microscope slides, stained with 0.01% (w/v) toluidine blue and observed under light microscope (Olympus, USA). Digital photographs were taken with a camera, and then cell area was measured with the 3D-Doctor software (Able Software Corp., Lexington MA, USA).

3.5.5. Starch quantification

Starch concentration was determined on 8 bulbs per treatment. Harvests for starch quantification occurred at the same time as for biomass measurements. Starch concentration was assayed according to the colorimetric method of Blakeney & Mutton (1980) and the extraction method Castonguay, Nadeau and Simard (1993). Bulbs were dried at 70°C, weighed, heated 20 min at 65°C in a solution of methanol, chloroform and water (12:5:3), grounded and centrifuged at 4°C. Starch contained in the pellet fraction was then gelatinized in boiling distilled water (90 min) and hydrolyzed at 55°C (1-h) using *Rhizopus* amyloglucosidase (Sigma-Aldrich, St-Louis, MO, USA). Reducing sugars from starch hydrolysis were quantified colorimetrically at 415 nm using *p*-hydroxybenzoic acid hydrazide (PAHBAH). Starch concentration was determined by comparison with a standard curve. Starch content was estimated as follow: value of starch concentration from each sample × mean dry mass value at the given time.

3.5.6. Nutrient analysis

N, P and K analysis were done on previously-grounded leaf of the eight plants used for growth measurements, pooled two by two, at the Laboratoire Daishowa, Pavillon de l'Envirotron, Université Laval. N and P concentrations were determined colorimetrically after digestion with sulphuric and selenic acid and hydrogen peroxide, and K concentration was determined by atomic absorption spectroscopy. Leaf nutrient content was calculated from the product of nutrient concentration by leaf mass.

3.5.7. Statistical analysis

Growth of *E. americanum* being in part governed by the field conditions of the preceding year, experiments of both years were analyzed separately as a complete randomized design and each plant was considered as a replicate. T-tests were performed to compare leaf phenological variables from leaf unfolding to leaf yellowing. Two-way ANOVAs were performed to compare the dependent variables: bulb, leaf and root dry weights, leaf area and starch and nutrient contents and concentrations, with time and treatment ($[CO_2]$) as main factors. Respiration rates were compared using two-way ANOVAs with date and treatment as factors while photosynthetic rates were compared with t-tests using the daily mean values due to the fact that photosynthetic measurements were not always recorded on the same days for both treatments. As gas exchange measurements have been taken at different times during the afternoon and early evening, linear regressions were performed to investigate whether assimilation rates changed with time during the day and could influence the comparisons between the two treatments. All statistical analyses were performed using the SAS 9.1 version (SAS Institute, Cary, NC, USA). *A posteriori* multiple comparisons tests were performed using Fisher's LSD to complete the analysis. A critical value of $\alpha=0.05$ was used for the tests of significance and data were transformed when they did not meet the criteria of normality and homogeneity of variance of the residues.

3.6. RESULTS

3.6.1. Leaf longevity

The concentration in CO_2 did not affect the duration of the green-leaf period of the plants (Fig. 3.1). In 2004, the yellowing of the distal part of the leaf occurred after 23.9 ± 1.3 days of growth under ambient [CO_2], and after 21.5 ± 1.8 days under high [CO_2] (t=-1.07; *P*=0.292). In 2005, plants grown under high [CO_2] began to yellow after 19.2 ± 0.9 days and control plants after 19.9 ± 1.0 days (t=-0.51; *P*=0.615).

In contrast, [CO_2] affected the leaf longevity. In 2004, plants grown under elevated [CO_2] lived on average 4 days more (40.3 ± 1.1 days) than control ones (36.1 ± 1.3 days; t=2.47; *P*=0.019). In 2005, no difference was found between both treatments (t=1.80; *P*=0.091); Plants grown in ambient [CO_2] died on average after 30.9 ± 0.9 days and those grown under elevated [CO_2] after 33.5 ± 1.1 days.

3.6.2. Plant growth

[CO_2] did not influence leaf area, leaf dry mass, and root dry mass (measured in 2004 only) in either year (Fig. 3.2). Leaf dry mass changed with time in 2004 ($F_{3,46}$=3.00; *P*=0.040) and in 2005 ($F_{2,26}$=7.75; *P*=0.002), as well as leaf area in 2004 ($F_{3,46}$=13.17; *P*<0.001). The root dry mass in 2004 and the leaf area in 2005 remained unchanged during the growing season.

In 2004 there was a significant interaction between time and treatment ($F_{5,71}$=2.59; *P*=0.033) for bulb mass, suggesting that the growth rate was different between the two treatments (Fig. 3.1a). During the two first weeks following leaf unfolding, the plants exhibited the same biomass increase. The differences appeared between Day 12 and the beginning of leaf yellowing, when control plants accumulated more biomass than those

grown under high [CO_2]. Then, whereas the bulb continued to grow during leaf yellowing at high [CO_2], no significant difference of bulb mass was found for control plants between the onset of leaf yellowing and complete leaf senescence. No significant difference between treatments was noticed in the final mean values of bulb mass.

In 2005 (Fig. 3.1b), the same tendency were observed, but time was the only factor that influenced significantly the growth of the bulb ($F_{5,54}$=199.29; P<0.001). The biomass of the bulb was stable during the first 4 days following leaf unfolding, and then it increased until leaf yellowing. A statistically significant raise in average bulb dry weight between half yellowing of the leaf and complete leaf senescence was noticed; this raise is likely due to the biomass increase of the bulbs at elevated [CO_2], because no changes in bulb biomass of the plant grown in ambient [CO_2] were visible.

3.6.3. Gas exchange measurements

Net assimilation rates were higher in plants grown under high [CO_2] than under ambient condition in both 2004 (t=6.95, P<0.001) and in 2005 (t=3.93, P=0.003). Mean assimilation rates were respectively 6.0 ± 0.3 and 11.4 ± 0.9 µmol m^{-2} s^{-1} in plants grown under ambient and under elevated [CO_2] condition in 2004, and respectively 16.0 ± 0.7 and 24.2 ± 0.2 µmol m^{-2} s^{-1} in 2005 (Fig. 3.3). There were no significant changes in net assimilation rates with time in plants grown under ambient condition ($P \geq 0.75$) and a slight increase in plants grown under elevated [CO_2] condition (r^2 = 0.15, P = 0.008 in 2004 and r^2 = 0.04, P = 0.06 in 2005). The differences observed between the two groups of plants were thus present throughout the day. Net assimilation rates were much higher in 2005 than in 2004 under both ambient and elevated [CO_2] conditions (fig. 3.3). However, total leaf area was much smaller in 2005 than in 2004 (fig. 3.2). Indeed, total carbon fixed per day was quite similar in 2004 and 2005 and higher under elevated [CO_2] condition than under ambient condition (data not shown).

Respiration rates were much greater in the bulb ($F_{1,28}$=14.91, P<0.001) when plants were grown under elevated [CO_2] than when grown in ambient condition. Under ambient conditions, mean respiration rate was respectively 5.16 ± 0.50 and 4.41 ± 0.34 nmol g FW^{-1} s^{-1} on Day 13 and 18. Under elevated [CO_2], mean values of 6.50 ± 0.51 and 6.59± 0.47 nmol g FW^{-1} s^{-1} were recorded for the same days. The mean values at Day 13 and 18 were not different ($F_{1,28}$=0.52, P=0.475). The mean leaf respiration rate was affected by the [CO_2] ($F_{1,26}$=49.49, P<0.001) and by time ($F_{1,26}$=75.17, P<0.001); the values of leaf respiration were 5.06 ± 0.28 and 3.00 ± 0.28 μmol m^{-2} s^{-1} in plants grown under ambient [CO_2], and 7.39 ± 0.30 and 4.60 ± 0.19 μmol m^{-2} s^{-1} for those grown under elevated [CO_2], respectively after 12 and 13 days following leaf unfolding, and mean values were greater on Day 13 than on Day 12.

3.6.4. Bulb starch storage

In 2004, the time × treatment interaction was significant for starch concentration ($F_{5,70}$=2.77; P=0.024) and starch content ($F_{5,70}$=55.01; P<0.001) in the bulb (Fig. 3.4). Maximal starch concentration was reached very rapidly (as soon as the second harvest). A sudden drop in starch concentration in the bulb of control plants was observed between half yellowing and complete leaf senescence. Otherwise, control plants showed slightly higher starch concentration than plants grown at high [CO_2] for most of the growth period. The curve of increase in bulb starch content was very similar to that of the increase in biomass (Fig. 3.1). Differences between the two treatments appeared about two weeks after leaf unfolding. At leaf yellowing, starch content was greater in bulbs grown under ambient condition, but the difference disappeared during leaf senescence.

In 2005, time was the only variable influencing the changes in bulb starch concentration ($F_{5,53}$=36.56; P<0.001) and bulb starch content ($F_{5,53}$=83.94; P<0.001; Fig. 3.4). Final starch concentration was reached between 5 and 10 days following leaf unfolding. Starch started to accumulate in the bulb after Day 4 and then the content increased until leaf were half yellowed. The two-way ANOVA did not show significant difference in starch content

between the two treatments, but similarly to what was observed in 2004, control plants had higher starch content than plants grown at elevated CO_2 concentration at the onset of leaf yellowing.

3.6.5. Nutrient status

In the leaf, no treatment effect was observed for N concentration in 2004 ($F_{1,30}$=0.00, P=0.954) and in 2005 ($F_{1,30}$=3.14, P=0.087; Fig. 3.5). N concentration decreased slowly, but regularly, with time ($F_{4,30}$=33.06, P<0.001 in 2004; $F_{4,30}$=113.52, P<0.001 in 2005), and the shape of the curves suggests that the mobilization was not greater once leaves started to senescence. N content in the leaf was significantly affected by the time x treatment interaction in 2004 ($F_{4,30}$=4.44, P=0.006). N content was relatively stable during the first days of growth, but slightly greater in plants grown under high [CO_2] during the growing season. In 2005, time was the only variable that influenced the foliar N content ($F_{4,30}$=81.50, P<0.001) and no significant content changes were observed before leaf yellowing.

In the bulb, N content was affected by a time × treatment interaction ($F_{5,36}$=8.47, P<0.001; Fig. 3.5). N content started to increase when leaf N content started to decrease, that is after Day 12 and up to complete leaf senescence. Average content was slightly greater in control plants when leaf started to senesce, then similar content were assessed during leaf senescence and the tendency was inverted for the last data point.

3.7. DISCUSSION

The results reported in this study demonstrated that *E. americanum* did not take advantage of an increase in CO_2 concentration. Net photosynthesis rates were much greater when plants were grown under high [CO_2] conditions than under ambient [CO_2] conditions (Fig. 3.3). But in contrast with a large number of species that respond to the improved carbohydrate supply at elevated [CO_2] through anatomical or physiological changes

(Kimball, 1983; Ceulemans & Mousseau, 1994; Jablonski, Wang & Curtis, 2002; Poorter & Navas, 2002), no positive effects were noticed on *E. americanum* growth and development. Leaf growth was not affected significantly by the treatment, as shown by leaf area and leaf dry weight values at maturity (Fig. 3.2). Both parameters increased with time, due to continued leaf growth. These findings contrast with the results of several other studies showing that C_3 plants generally exhibit greater shoot production in response to elevated [CO_2] (Ceulemans & Mousseau, 1994; Taylor *et al*., 1994; Pritchard *et al.*, 1999). But our results are consistent with the view that enhanced [CO_2] tend to be less favourable to non-leguminous plants (Ainsworth & Long, 2005), and particularly wild species (Hunt *et al*., 1991; Wolfenden & Diggle, 1995; Jablonski, Wang & Curtis, 2002) than to leguminous plants and trees. No difference was observed on foliar N concentration between the two treatments, suggesting that leaves did not acclimate to elevated [CO_2].

Surprisingly, elevated [CO_2] did not enhance the growth of the belowground parts. Indeed, neither roots (Fig. 3.2) nor bulb (Fig. 3.1) final biomasses were significantly affected by the treatment, whereas such responses are frequently reported in the literature, both on roots (Rogers *et al.*, 1992, 1994) and on perennial organs (Daymond *et al.,* 1997; Miglietta *et al*., 1998; Sicher & Bunce, 1999; Chen & Setter, 2003). Concerning the root system, the lack of response is likely due to the fact that root growth in *E. americanum* is initiated early during autumn and growth mainly occurs during the cold season, with very limited growth in spring (Brundrett & Kendrick, 1988). Increased yields and larger bulbs at high [CO_2] were reported in onion (Daymond *et al.,* 1997), and increased tuber size due to increased cell number rather than cell volume was also demonstrated in potato (Chen & Setter, 2003). No similar pattern was observed here. No change in cell size (data not shown) was noticed at elevated [CO_2], and given that the final bulb biomass was not different between the two treatments, it is unlikely that [CO_2] influenced bulb cell development. Of the many responses of plants to elevated [CO_2], a lack of impact on growth of main plant sink organ(s) is rarely reported, because plants are generally capable (when nutrients are not limiting) to modulate the growth of their organs in response to their photosynthetic status. Such a phenomenon may, nonetheless, occur in spite of elevated [CO_2] when plants are sink-limited (Paul & Foyer, 2001; Woodward, 2002), as shown in onion and potato where

similar rates of biomass accumulation are reported from bulbing to bulb maturity (Daymond *et al.*, 1997), and before tuber initiation (Conn & Cochran, 2006), respectively, under normal and high [CO_2] conditions.

In the studies of Daymond *et al.* (1997) on onion and Conn & Cochran (2006) on potato, the sink-limited developmental stage was characterized by a temporary adjustment of the net photosynthesis rate. A similar adjustment did not seem to occur here (maybe towards the end of the mature phase during the second year experiment), since net photosynthesis rates of *E. americanum* stayed high at elevated [CO_2] (Fig. 3.2). However, our results clearly indicated that elevated [CO_2] neither accelerated bulb growth, nor bulb starch storage (Fig. 3.4). As a result, elevated [CO_2] had little impacts on leaf phenology: leaf yellowing was synchronous between the two treatments, in both 2004 and 2005, and complete leaf senescence occurred at the same time in both treatments in 2005, while it was four-day delayed at elevated [CO_2] in 2004 (Fig. 3.1). However, there might be some kind of adjustment of the net assimilation rates according to the size of the leaf, since the smaller leaves of 2005 had much higher net assimilation rates than the larger leaves of 2004. Leaf duration being not reduced under higher [CO_2], the data do not support our hypothesis.

Since the greater carbohydrate supply at elevated [CO_2] resulted in neither leaf storage (based on leaf biomass), nor growth and C storage in the bulb, another pathway of C use has to be modulated. Indeed, increased respiration rates at elevated [CO_2] did not result in better plant growth, and no indications allow us to conclude that plant-tissue maintenance was significantly greater for that treatment since nitrogen bulb and leaf contents were not, or only slightly, different between the two treatments (Fig 3.5). As a result, the respiratory C loss under high [CO_2] suggests a decreased respiration efficiency, a phenomenon that generally occurs when electron transport chain is not coupled to proton pumping (Lambers, Chapin & Pons, 1998).

We hypothesize that high [CO_2] enhanced the activity of alternative respiration pathways. One of these pathways might be the cyanide-insensitive respiration, which is known to be

present in numerous species (Azcón-Bieto, Lambers & Day, 1983), for instance in tulip (Kanneworff & van der Plas, 1994) and in iris (Marissen, Kanneworff & van der Plas, 1991). The biological functions of non-phosphorylating pathways, as well as their regulation are not well known but several studies have demonstrated their implication in response to environmental changes (Breidenbach *et al.*, 1997; Gonzalez-Meler *et al.*, 2004; Sieger *et al.*, 2005). Increased respiratory losses support the hypothesis that growth of *E. americanum* is more limited by bulb growth capacity, and thus sink activity, than by C uptake (Lapointe, 2001). This species was not capable of increasing the strength of its sinks, no more than to acclimate its leaves, in response to greater CO_2 availability. As a result, increased respiratory losses, via alternative pathways, appeared as a good mean to avoid starch accumulation in the leaf (Lambers, 1982; Azcón-Bieto, Lambers & Day, 1983) and damaging metabolic changes (Paul & Foyer, 2001; Rolland, Moore & Sheen, 2002), which could even induce earlier leaf senescence and smaller final bulb size.

In summary, these results indicated that an increase in CO_2 has no effect on the growth of this species. The plants exhibited a general lack of plasticity in response to CO_2 availability. Leaf morphology did not change with [CO_2] and the growth of the bulb, which is the main sink of the plant, and its final biomass and starch content and concentration were not positively influenced by increased CO_2 availability. *E. americanum* bulb growth thus appears to be more limited by cell growth capacity than by C availability at least under high light and warm temperature conditions. Despite no change in growth rate, photosynthetic activity under elevated [CO_2] was not down-regulated and no reduction in N concentration was observed. But the higher leaf and bulb respiration rates exhibited by the plants grown at higher [CO_2] might explain where the surplus of C went and thus that no acclimation or negative feedback were observed. These findings corroborate the hypothesis of a growth regulation by sink strength in this species.

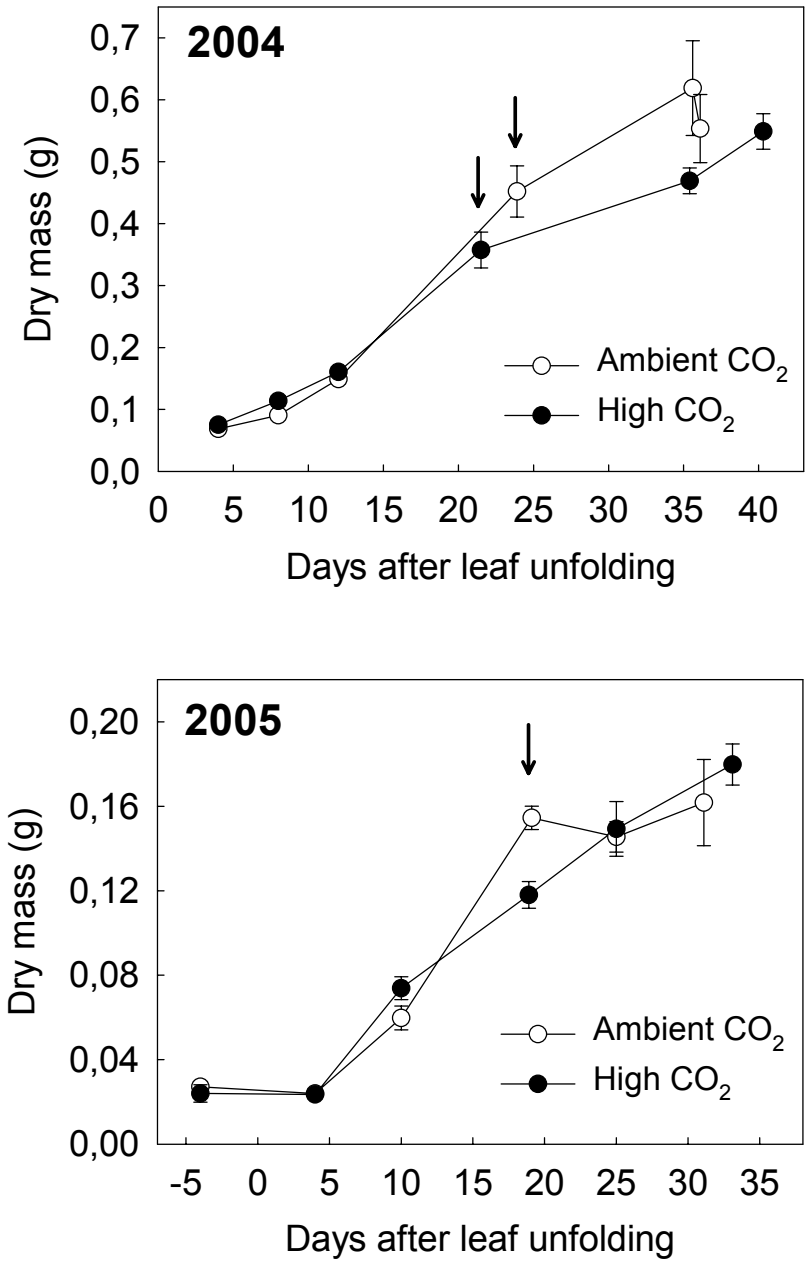

Figure 3.1. Variation in the mass of *E. americanum* bulbs during the epigeous growth period, in plants grown at ambient (open symbols) and high [CO_2] (closed symbols) in 2004 (above) and 2005 (below). Means ± 1 SE are shown (*N*=7 in 2004 and *N*=6 in 2005). The arrows indicate the onset of leaf yellowing.

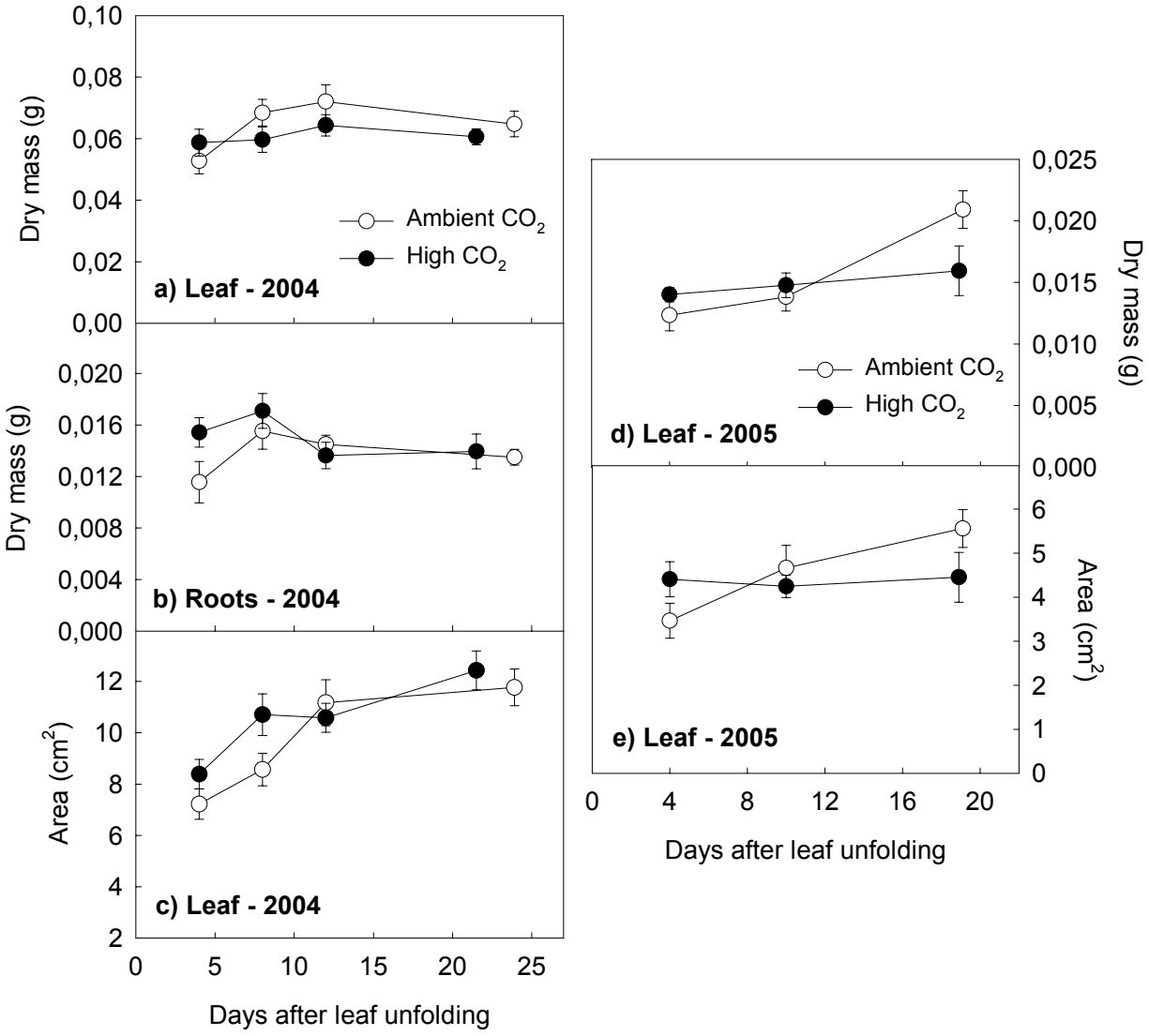

Figure 3.2. Time course of leaf (a, d) and root (b) mass, and leaf area (c, e) from Day 4 following leaf unfolding to the onset of leaf yellowing, for *E. americanum* plants grown at ambient (open symbols) and high [CO_2] (closed symbols) in 2004 (a, b and c) and in 2005 (d and e). Means ± 1 SE are shown (*N*=7 in 2004 and *N*=6 in 2005).

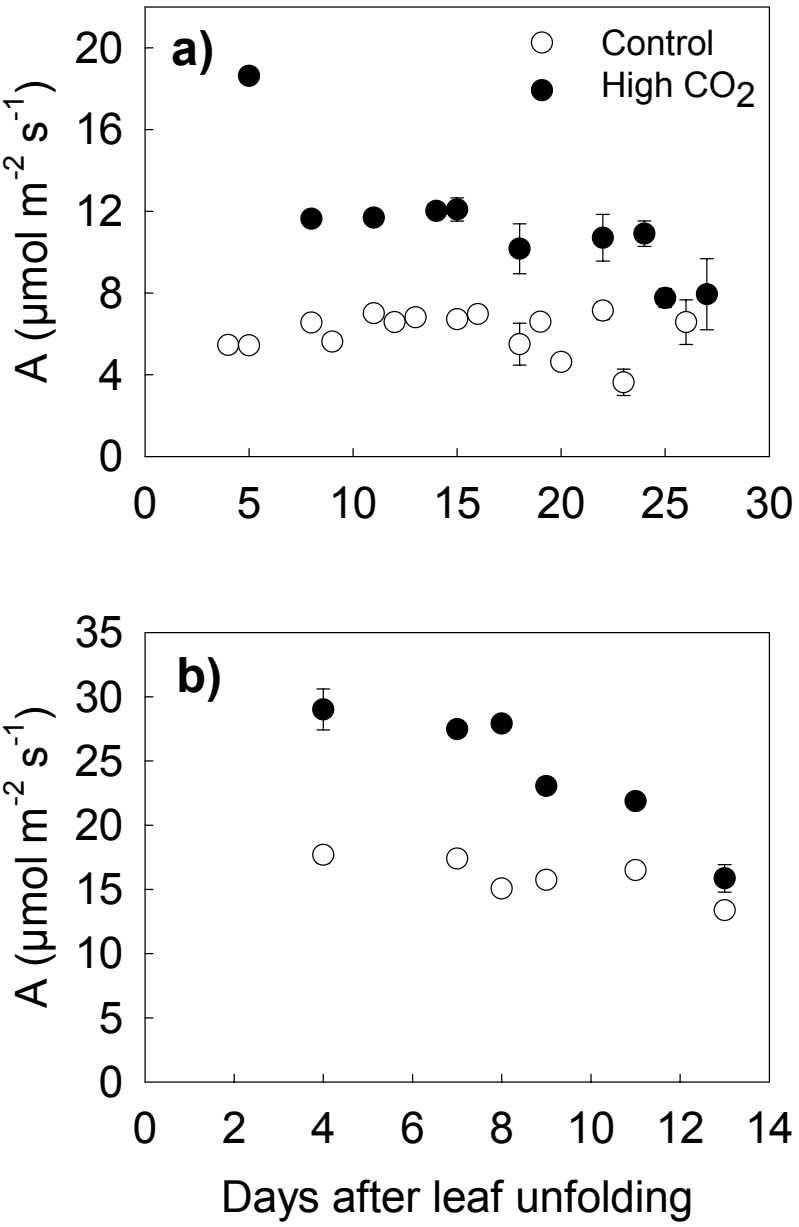

Figure 3.3. Time course of the net photosynthesis rate per leaf area a) in 2004 from day 4 after leaf unfolding to the onset of leaf yellowing and b) in 2005 during the first two weeks after leaf unfolding, for *E. americanum* plants grown at ambient (open symbols) and elevated CO_2 concentrations (closed symbols). Means ± 1 SE are shown (*N*=1 to 12).

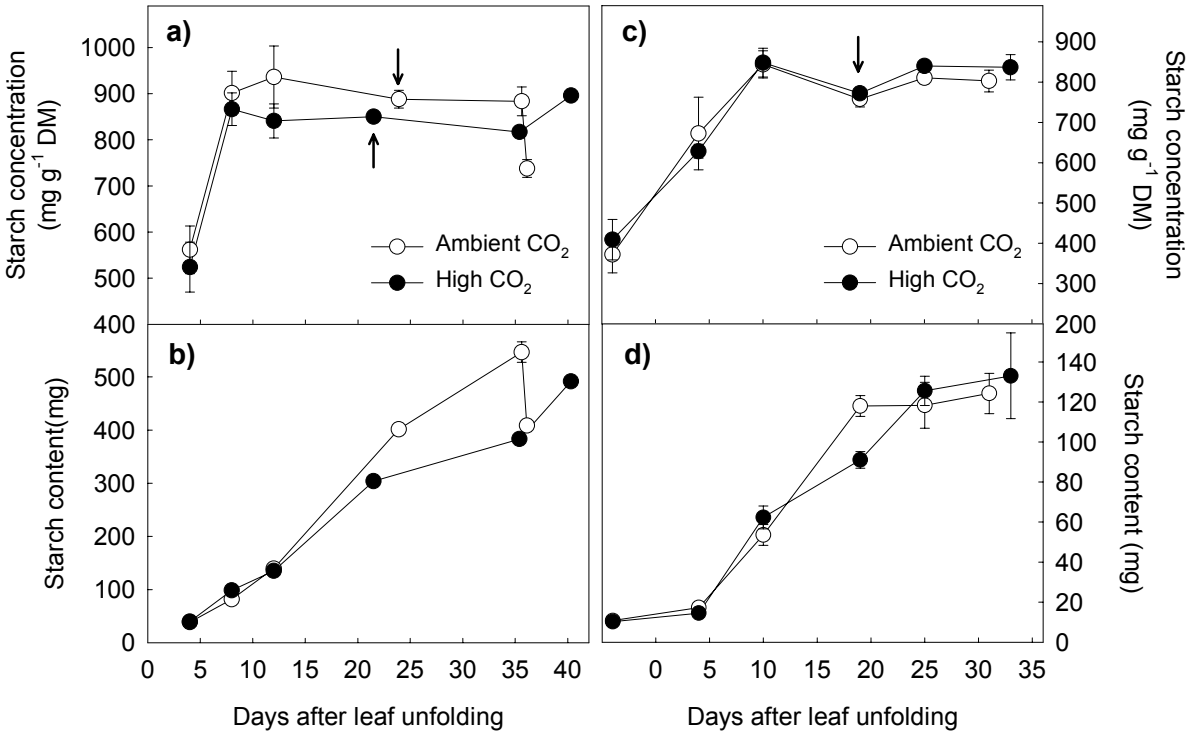

Figure 3.4. Time course of starch concentration (a, c) and starch content (b, d) in the bulb of *E. americanum* during the epigeous growth period. Data originate from plants grown at ambient (open symbols) and high CO_2 concentrations (closed symbols) in 2004 (a, b) and in 2005 (c, d). Means ±1 SE are shown (*N*=7 in 2004 and *N*=6 in 2005). The arrows indicate the onset of leaf yellowing.

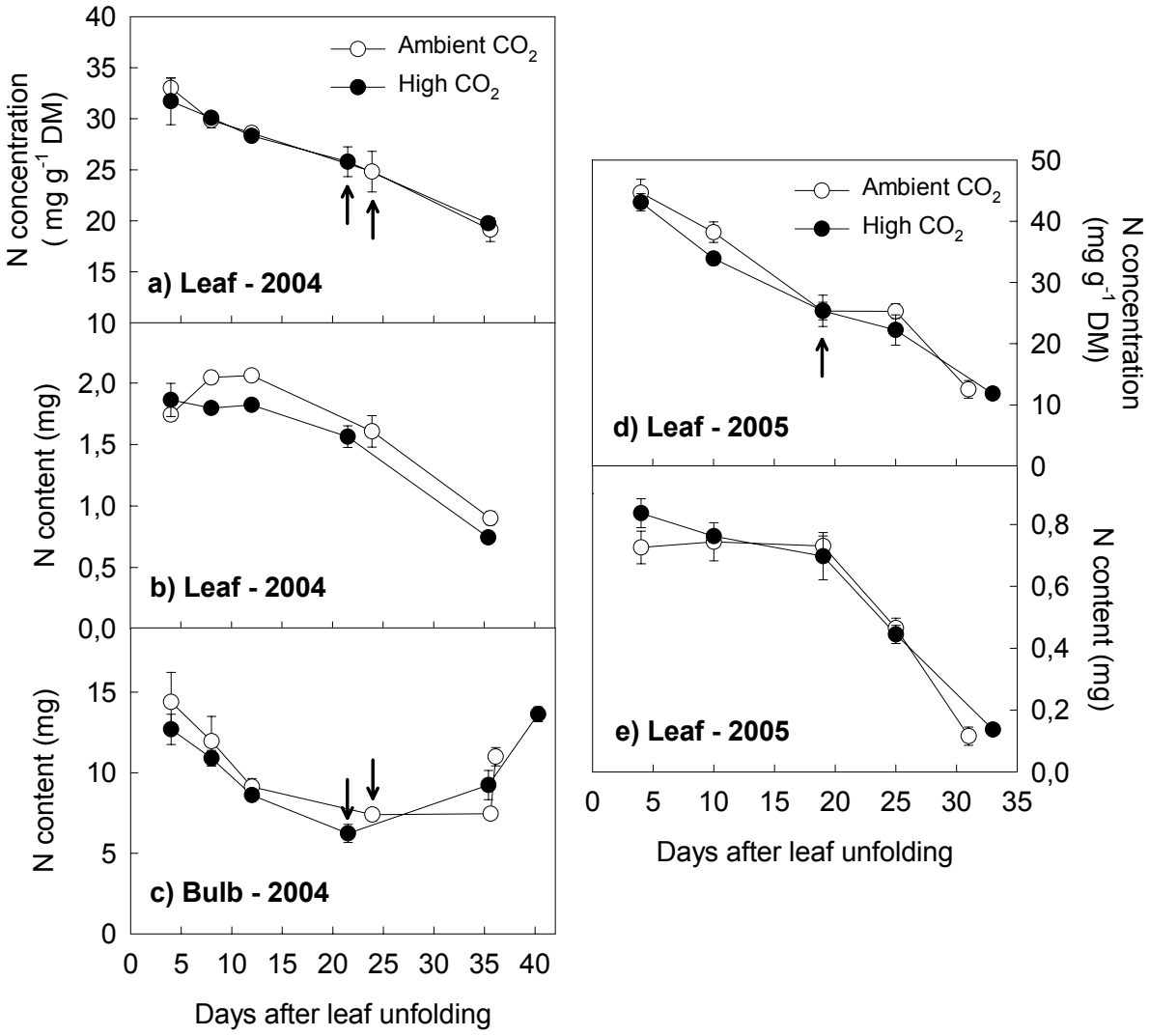

Figure 3.5. Time course of foliar N concentration (a, d), foliar N content (b, e) and bulb N content (c) for *E. americanum* plants cultivated at ambient (open symbols) and elevated [CO_2] (closed symbols) in 2004 (a, b, c) and 2005 (d, e). Means ± 1 SE (*N*=4) are shown from leaf unfolding to half leaf senescence (Harvests 1 to 5), except for bulb N content where data from completely senesced plants is also presented. The arrows indicate the onset of leaf yellowing.

Conclusion générale

La présente étude avait pour but d'investiguer plus en profondeur les relations source/puits de C chez les plantes éphémères printanières pérennes en prenant l'érythrone d'Amérique, une espèce indigène des forêts décidues d'Amérique du Nord, comme modèle. Nous sommes partis des résultats d'une étude récente (Lapointe & Lerat, 2006) qui soulignait le fait que des températures élevées avaient un impact négatif sur la croissance de l'organe souterrain ainsi que sur la durée de vie des parties aériennes de l'érythrone d'Amérique, et nous nous sommes demandés s'il était possible de mettre en évidence une relation entre la durée de vie des parties aériennes (source de C) et la durée de remplissage de l'organe de réserve (puits principal de C). Notre hypothèse principale était que le maintien de la force des puits de C est le facteur déterminant la durée de vie de la feuille chez cette espèce. Dans un premier temps, nous nous avons tenté de caractériser plus en détails l'impact de la température sur la croissance de l'érythrone afin de savoir si la sénescence prématurée de la feuille à 18/14°C était due à la croissance plus faible du bulbe à cette température, et donc que le signal de la sénescence venait des parties souterraines et non des parties aériennes. Dans un deuxième temps, l'augmentation de la concentration en CO_2 de l'air dans les chambres de croissance devait nous permettre, via une augmentation du taux de photosynthèse net des plantes, d'augmenter la force des sources de C afin d'en étudier les répercussions sur la vitesse de remplissage du bulbe.

Dans la littérature, rares sont les études qui relatent un effet négatif d'une augmentation de température de quelques degrés par rapport aux normales saisonnières chez les plantes de milieux « extrêmes », telles que les plantes arctiques et alpines. Les espèces vivant dans ces milieux en tirent généralement un bénéfice, ne serait-ce que pour investir davantage de ressources pour la reproduction. Chez l'érythrone d'Amérique, il a été montré que l'augmentation de température réduit la durée de vie de la feuille ainsi que la taille finale du bulbe. Cependant, les récoltes séquentielles nous ont permis de constater que la température n'a pas un effet aussi marqué que ce que nous pouvions penser. Le taux de croissance des plantes est faiblement affecté, si bien qu'avant le début de la sénescence de la feuille à

18/14°C, il est très difficile de discerner les plantes des deux traitements. Une telle observation est probablement liée au fait que le taux de photosynthèse net n'est pas affecté. La quantité d'amidon synthétisée par les plantes est similaire, et la croissance du bulbe par élongation cellulaire et non par division cellulaire (une stratégie relatée par Grime (1983) chez les espèces exposées aux températures froides) favoriserait alors un gain de biomasse très semblable pendant un certain temps aux deux températures. D'après ces résultats, il est légitime de penser que les effets de la température observés lorsque les plantes sont comparées après sénescence complète de la feuille sont dus principalement à une réduction de la période où la feuille est photosynthétiquement active à 18/14°C. La remobilisation simultanée des nutriments de la feuille vers le bulbe aux deux températures, qui induit une concentration supérieure en nutriments dans les feuilles à 18/14°C au moment du jaunissement, démontre qu'au régime de température élevée, les plantes vivent moins longtemps, et que l'accélération du cycle de croissance n'en est pas la cause.

La sénescence étant un processus complexe, il est difficile de bien le caractériser à partir d'un nombre limité de variables. En se basant sur la teneur en nutriments des feuilles, il semble que l'érythrone d'Amérique présente non pas une accélération de la sénescence telle qu'observée chez le crocus (Badri *et al.*, soumis), ou chez l'érythrone du Japon Yoshie & Fukuda, 1994) mais plutôt une sénescence précoce de la feuille. Chez le crocus, la teneur en nutriments commence à diminuer plus tôt aux températures élevées. Chez l'érythrone du Japon, toutes les étapes de la vie de la feuille – émergence, développement, maturité et sénescence – sont accélérées aux températures plus élevées. D'autres mesures du processus de sénescence telle la destruction des chloroplastes et l'activation de certains gènes seraient nécessaires pour confirmer notre hypothèse d'une sénescence induite de façon précoce plutôt que de façon accélérée chez l'érythrone d'Amérique aux températures plus élevées.

Augmenter la teneur de CO_2 dans l'air était une approche intéressante pour tester d'une autre façon l'hypothèse de limitation de la croissance par les puits. En effet, il suffisait que le CO_2 n'ait pas d'effet sur les processus de division et d'élongation cellulaire et que l'augmentation de CO_2 entraîne une augmentation de l'assimilation nette des plantes (phénomène couramment rapporté dans la littérature) pour que, théoriquement, le bulbe se

remplisse plus vite et que la saturation du puits en C provoque la sénescence prématurée des parties aériennes. Cependant, le résultat fut tout autre. Durant les deux années d'étude, le surplus de sucres formé suite à l'augmentation de la concentration de CO_2 ne put profiter aux plantes. La durée de vie de la feuille ne fut pas réduite, et une des deux années, les bulbes accusèrent même un retard significatif de croissance, malgré le taux de photosynthèse net plus élevé. Ces résultats suggèrent que la vitesse de remplissage du bulbe en amidon est déterminée par la vitesse d'élongation des cellules. Ensuite, plusieurs points nous indiquent que cette espèce présente une très faible plasticité, adoptant une stratégie de « ça passe ou ça casse ». La remobilisation des nutriments peut avoir lieu tôt dans la saison de croissance, alors que la plante est en pleine croissance ; la conductance stomatique est peu régulée, aussi bien lors d'une augmentation de la température (et donc de la transpiration) que d'une augmentation de [CO_2] (données non présentées) ; l'appareil photosynthétique ne s'acclimate pas à l'augmentation de CO_2 (aucune diminution de la concentration foliaire d'azote ne fut observée) contrairement à ce qui est souvent rapporté dans la littérature, et la plante est alors condamnée à « brûler » ses surplus de sucres, possiblement via un recours aux voies alternatives respiratoires, pour éviter tout dommage cellulaire et les rétroactions négatives.

Pour conclure, cette étude nous a permis de mieux comprendre la croissance en général de la plante, mais aussi de vérifier certaines observations d'autres travaux. Il existe à ce jour très peu de données dans la littérature concernant la réponse des espèces végétales indigènes non utilisées en agriculture ou ligniculture face aux changements climatiques, dont deux composantes sont les changements de température et de concentration de CO_2 dans l'air. Nous apportons alors indirectement des données supplémentaires sur cette problématique, qui sont d'autant plus intéressantes que l'on sait, par exemple dans le cas du CO_2, que la réponse des plantes peut être très variable suivant l'espèce considérée (Poorter & Navas, 2002). Ces données pourront servir à titre de comparaison dans le cas d'études portant sur les plantes printanières, les plantes de « milieux froids » ou encore sur les plantes à bulbes. Des tests de « levées de dormance » afin d'accélérer la croissance de la feuille pendant la période de froid et les tentatives de réduction celle-ci, qui est d'environ 5 mois pour que la feuille pointe spontanément à la surface (réalisés avec P. Jobin), nous ont

également permis de constater que le cycle de l'érythrone d'Amérique est difficile à modifier. Par contre, cette espèce supporte très bien la culture en chambre de croissance.

Maintenant, diverses avenues mériteraient d'être explorées afin de compléter cette étude. Par exemple, il serait intéressant d'étudier plus précisément les processus d'élongation et de division cellulaire du nouveau bulbe, dès sa formation. Un suivi sur quelques années, en plus du décompte final de la taille et du nombre de cellules, permettrait de mieux caractériser les effets de l'environnement et peut-être d'expliquer les variations interannuelles de croissance des plantes. Ensuite, il serait intéressant de réaliser une autre répétition de l'expérience sur les effets de la température (chapitre 2) afin de confirmer les effets sur la division et l'élongation des cellules du bulbe. Si toutes les difficultés techniques étaient mises de côté, l'idéal pourrait être de mettre au point une expérience directement sur le terrain, par exemple, via l'utilisation de chambres adaptées (*Open Top Chambers*), afin de confirmer nos observations. Il serait également intéressant de regarder s'il est possible de mettre en évidence une augmentation de la concentration de certains sucres (même minime) dans les feuilles au moment où le bulbe arrête de croître. Ces mesures pourraient être plus précises que les mesures de photosynthèse pour détecter le moment où la feuille reçoit le signal de la sénescence. Concernant l'expérience sur les effets de la concentration en CO_2, l'étude des mécanismes respiratoires en condition de [CO_2] élevée (recours aux voies alternatives de respiration ?) apporterait beaucoup pour la compréhension de la physiologie de cette espèce. Finalement, un point qu'il serait intéressant d'investiguer, à la fois dans l'expérience de la température et celle de la concentration en CO_2, serait la prise des mêmes mesures sur des individus qui se reproduisent de façon sexuée. On pourrait alors utiliser le fruit comme un puits de C supplémentaire et ainsi comparer des individus produisant un fruit à des individus dont on l'aurait supprimé.

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