Rôle du métabolisme carboné dans la modulation des relations source-puits et études des facteurs impliqués dans l'induction de la sénescence foliaire chez une éphémère printanière (Erythronium americanum)

Thèse

Yanwen Dong

Doctorat en biologie
Philosophiæ doctor (Ph. D.)

Québec, Canada

Rôle du métabolisme carboné dans la modulation des relations source–puits et études des facteurs impliqués dans l'induction de la sénescence foliaire chez une éphémère printanière (*Erythronium americanum*)

Thèse de doctorat :

Université Laval, Canada

Yanwen Dong

Sous la direction de :

Line Lapointe, directrice de recherche

Dominique Gérant, codirectrice de recherche

Département de biologie

Faculté des sciences et de génie

Université Laval

Québec

Résumé

Certaines géophytes telles que les éphémères printanières sont connues pour mieux se développer à des températures basses, avec comme résultat de plus gros organes pérennes souterrains. Une température plus basse induit une plus longue longévité des feuilles, ce qui permet une plus grande quantité du carbone fixé qui est alloué dans le bulbe et investi principalement sous forme de stockage (amidon), ce qui augmente la quantité de réserves par rapport à une température plus élevée. Les travaux précédents suggèrent que cette croissance accrue à basse température est liée à un meilleur équilibre entre l'activité de l'organe source et celle du puits, ce qui expliquerait la durée de vie plus longue des feuilles. Dans cette étude, nous voulions approfondir notre compréhension des facteurs intrinsèques qui influencent la croissance des organes de réserve chez les géophytes et qui expliquent les fortes croissances observées aux faibles températures chez ces espèces. Nous tentions également d'identifier les voies signalétiques qui induisent la sénescence foliaire lorsque le puits se remplit d'amidon en ayant recours à une approche métabolomique et à l'établissement du profil phytohormonal. L'espèce étudiée, l'érythrone d'Amérique (*Erythronium americanum* Ker-Gawl.), a été cultivée suivant trois régimes de températures: 8/6 °C, 12/8 °C et 18/14 °C (jour/nuit). Les taux respiratoires des plantes ont été mesurés à la température de croissance et à une température commune afin de tester notre hypothèse selon laquelle la respiration s'acclimate à la température de croissance tant au niveau de la feuille que du bulbe, principalement par la voie alternative, comme moyen de réduire le déséquilibre source−puits. Les différents glucides non structuraux (NSC) et structuraux (SC) du bulbe ont été déterminés qualitativement et quantitativement, ce qui nous a permis de vérifier si les plantes pouvaient ajuster leur répartition du carbone dans différents composés (NSC *vs* SC) une fois que les cellules sont remplies d'amidon. Nous avons également caractérisé les phytohormones et métabolites et surtout ceux qui sont étroitement associés au stade phénologique précédant la sénescence foliaire afin d'identifier les voies signalétiques qui établissent le lien entre la diminution de la force du puits et l'induction de la sénescence foliaire. L'homéostasie des taux de respiration au niveau de la feuille, combinée à un faible taux d'assimilation chez les plantes cultivées à basse température suggère que ces plantes sont capables de réduire la quantité de carbone disponible pour la

translocation vers le bulbe afin de maintenir un meilleur équilibre entre l'activité de la source et celle du puits, et ce pour une durée plus longue. La respiration du bulbe est stimulée au fur et à mesure que la limitation du puits se développe, répondant ainsi fort probablement à un déséquilibre source–puits. Les plantes cultivées à la température plus élevée semblent investir davantage de carbone vers les composants de la paroi cellulaire par rapport à celles cultivées à la température plus basse une fois que les cellules sont remplies d'amidon. Cet ajustement de la répartition du carbone entre NSC et SC pourrait permettre à ces plantes de maintenir leur force du puits du moins pendant quelques jours de plus. Certains métabolites et phytohormones semblent être des déclencheurs de la sénescence foliaire, mais plusieurs sont spécifiques à l'un ou l'autre des organes ou spécifiques à un régime de température. Un accroissement des niveaux de cytokinines lorsque la feuille devient mature et leur maintien à des valeurs élevées jusqu'au début de la sénescence foliaire pourrait contrebalancer l'abondance accrue des sucres solubles au moins pendant un certain temps et éviter ainsi d'induire une sénescence précoce. Nous avons également identifié cinq métabolites qui pourraient servir en tant que facteurs signalétiques généraux pouvant induire la sénescence foliaire, à savoir le 2-O-glycérol-β-D-galactopyranoside, le mannose, le fructose, le sorbose et le maltose. Cette étude nous a aidés à mieux caractériser les voies signalétiques qui associent la diminution de la force du puits et l'induction de la sénescence foliaire et à comprendre l'acclimatation de cette espèce à la température. Nous pouvons finalement conclure que cette géophyte printanière, dont la croissance est limitée par le puits, semble capable de moduler sa force de puits de manière différentielle sous différentes températures de croissance afin d'éviter la sénescence foliaire encore plus précoce dans les situations de déséquilibre source–puits.

Abstract

Some geophytes such as spring ephemerals are known to grow better at lower temperatures, which results in larger underground perennial organs. A lower temperature induces a longer leaf life span, which allows the plant to fix more carbon. This extra carbon is allocated to the bulb and invested mostly as storage (starch) form, thus increasing the amount of reserves found in cool compared to higher temperature grown plants. Previous works suggested that such increased growth at low temperature is related to a better equilibrium between source and sink activities. In this study, we wanted to deepen our understanding of the intrinsic factors that influence the growth of reserve organs in geophytes and to explain the strong growth observed at low temperature in these species. We also attempted to identify the signaling pathways that induce leaf senescence when the sink organ is filled with starch, using a metabolomic approach and a phytohormonal profiling. The species studied, yellow trout-lily (*Erythronium americanum* Ker-Gawl.), was grown at three temperature regimes: 8/6 ℃, 12/8 ℃ et 18/14 ℃ (day/night). Respiratory rates at both the leaf and bulb levels were measured at the respective growth temperatures and at a common temperature in order to test our hypothesis that dark respiration acclimates to growth temperature, mainly via the alternative pathway, as a means of reducing the source−sink imbalance. The different non-structural carbohydrates (NSC) and structural carbohydrates (SC) in the bulb were qualitatively and quantitatively determined, which allowed us to verify if the plants could adjust their carbon partitioning between different compounds (NSC *vs*. SC) once the cells are filled with starch. We also characterized phytohormones and metabolites, especially those closely associated with the phenological stages that precede senescence, to identify the signaling pathways that establish the link between the decrease of sink strength and the induction of leaf senescence in this species. Homeostasis of leaf respiration combined with lower assimilation in cool-grown plants suggests that these plants can reduce the amount of carbon available for translocation to the bulb to maintain a better balance between source and sink activities over a longer period. Bulb respiration can be stimulated as sink limitation builds up, likely in response to source–sink imbalance. A preferential carbon partitioning into cell wall compounds was found in warm-grown plants once the cells were filled with starch. Such adjustment of C between NSC and

SC could represent an effective way to maintain the sink strength under warm temperature at least for a few more days. Some phytohormones and metabolites appear to pay a role in triggering leaf senescence, but many are specific to one organ or specific to a temperature regime. Increased levels of cytokinins during the mature leaf stage and into the senescence stage might counteract the increasing abundance of soluble sugars at least for a while, and thus avoid inducing an early leaf senescence. We have also pointed out five metabolites that could potentially serve as general signaling factors to induce leaf senescence, namely 2-O-glycerol-β-D-galactopyranoside, mannose, fructose, sorbose and maltose. This study has helped us better characterize the signaling pathways that associate the decrease in sink strength with the induction of leaf senescence. It also improve our understanding of the thermal acclimation of this species. We may ultimately conclude that this sink-limited spring geophyte seems to be able to differentially modulate its sink strength under different growth temperatures in order to avoid early leaf senescence in situations of source–sink imbalances.

Table des matières

Liste des abréviations

2-iP: 2- isopenténylad énine;

A: assimilation nette;

ABA: acide abscissique;

ACC: acide 1-aminocyclopropane-1-carboxylique;

AOX: oxydase alternative;

C: carbone;

CKs: cytokinines;

DHZ: dihydroz éatine;

DHZR: dihydroz éatine riboside;

DM: masse s èche;

ESI-MS: Électrospray-spectrom étrie de masse;

GAs: gibb érellines;

GABA: acide γ-aminobutyrique;

HPAE-PAD: chromatographie ionique coupl ée à l'ampérométrie puls ée;

HEPES: acide 4-(2-hydroxy éthyl)-1-pip érazine éthane sulfonique;

IAA: acide indole 3-ac étique;

iPA: isopent ényl ad énosine;

JA: acide jasmonique;

KCN: cyanure de potassium;

MES: acide 2-(N-morpholino) éthanosulfonique;

N: azote;

NSC: glucides non-structuraux;

PPFD: densit é de flux photonique photosynth étique;

R_T: respiration totale à l'obscurité;

R_{alt}: capacit é de la voie respiratoire alternative;

R_{cyt}: capacit é de la voie respiratoire cytochromique;

RH: humidit é relative;

SA: acide salicylique;

SC: glucides structuraux;

SEM: erreur type;

SHAM: acide salicylhydroxamique;

SS: sucres solubles;

TCA cycle: cycle de l'acide tricarboxylique (également appelé cycle de l'acide citrique ou cycle de Krebs);

UPLC: chromatographie en phase liquide ultra-performante;

VPD: déficit de pression de vapeur;

Z: *trans*-zéatine;

ZR: *trans*-zéatine riboside.

Liste des tableaux

Liste des figures

Liste des annexes

À mon amour et mes parents

"There are no shortcuts to any place worth going."

- Beverly Sills

Remerciements

Je tiens à remercier chaleureusement, en premier lieu, ma directrice de recherche Line Lapointe, et ma co-directrice Dominique Gérant, pour leur confiance, patience et encouragement, pour toutes les compétences qu'elles m'ont transmises tout au long de ces années de recherche et pour leur aide précieuse dans la rédaction du rapport et la préparation de la présentation orale. Je remercie également mon co-directeur retraité Pierre Dizengremel, merci Pierre de m'avoir permis d'avoir eu cette opportunité d'explorer l'autre côté du monde.

Je voudrais remercier sincèrement les membres de mon jury, à savoir Dominique Michaud, Steeve Pepin et Guy Samson, pour avoir accepté d'évaluer cette thèse de doctorat et pour leurs précieux commentaires et suggestions. Mes remerciements s'adressent aussi à Conrad Cloutier pour ses commentaires sur mes résultats en tant que membre de mon comité d'encadrement.

Je remercie grandement l'ensemble du personnel de Département de biologie de l'Université Laval, de l'UMR 1434 SILVA Université de Lorraine, aussi bien à l'INRA qu'à la faculté, pour leur accueil sympathique et leur coopération professionnelle.

Un grand merci spécial à tous les collègues qui m'ont aidée dans la récolte des plantes sur le terrain, la mise en place et l'utilisation des appareils de mesure, des chambres de croissance et l'obtention des résultats: François Larochelle, Moustafa A. Khalf, Nicolas Martin, Julie Bussières, Pierre-Paul Dion, Marie-Hélène Jacques, Jade Boulanger-Pelletier (*R.I.P. chère Jade, ton sourire me manque tellement*), Dominique Manny et Karolane Pitre. Merci aussi à Gaétan Daigle pour l'aide précieuse apportée à nos analyses statistiques. Un grand merci à toutes les personnes que j'ai rencontrées et avec qui j'ai travaillé, pour leur aide dans la réalisation de ce projet dans les meilleures conditions et en répondant à mes questions diverses: à Québec: Marie-Pier Denis, Belén Merelas Meijide, Hugo Bertrand, Josée Pelletier, Diane Gagnon du Centre de recherche STELA/INAF, Marie-Claire Goulet du Laboratoire du Dr. Dominique Michaud, Édith Tousignant du Pavillon Envirotron, et

aussi toutes les personnes du secrétariat du Département de biologie; à Nancy: Stephane Desobry, Jean-Marc Guehl, de l'UMR Silva, et Christian Hossann du pôle isotope à l'INRA.

Merci à tous les amis chinois que j'ai rencontrés à Québec, nos soirées de cuisine de temps en temps qui me permettaient de ne pas trop me sentir dépaysée, merci d'avoir rendu ma vie plus heureuse ici: Chen Chen, Jing Wang, Wei Li, Tengfei Xu, Changhua Fang, Fangru Meng, Qiuying Liang, Huan Liang, Ye Kang, Ying Wang, Jingjing Ju, Jingqi Shi.

Je tiens à remercier tout particulièrement toute ma famille. Papa, Maman, je suis là grâce à votre amour, votre soutien et votre confiance sans faille. Merci d'avoir facilité ma vie. Merci d'avoir toujours cru en moi et de m'avoir soutenue dans toutes les décisions que j'ai prises.

Enfin, gros merci à mon amour Xiangtao. Merci d'être entré dans ma vie. Tu m'écoutes quand je te raconte mes soucis, malgré la distance géographique et le décalage horaire. Tu m'encourages tout le temps et tu es toujours là pour moi. Tu as quitté ton travail bien rémunéré sans aucune hésitation pour m'accompagner à Québec durant la période bien sombre de ma vie. Merci pour ta compagnie qui a permis d'éviter que la dépression et le désespoir ne m'envahissent. Merci mon cœur pour tout ce que tu fais pour moi.

Avant-propos

Cette thèse est rédigée sous forme d'articles scientifiques (Chapitre 1 à 3) et comporte, en outre, une introduction et une conclusion générale. Les travaux ont été menés en alternance entre le Département de biologie de l'Université Laval à Québec, et l'UMR 1434 SILVA Université de Lorraine/INRA à Nancy en France pendant les trois premières années. Les contributions respectives sont détaillées ci-après:

Chapitre 1. Yanwen Dong, Dominique Gérant et Line Lapointe sont les co-auteurs de cet article. J'ai réalisé l'ensemble des travaux et des analyses statistiques menant aux résultats présentés dans ce chapitre. J'ai effectué la rédaction de cet article sous la supervision de ma directrice de recherche Line Lapointe et de ma co-directrice de recherche Dominique Gérant. L'article a été soumis le 12 septembre 2017 et accepté le 7 novembre 2017 dans la revue *Botany* (vol. 96:97-109).

Chapitre 2. Yanwen Dong, Dominique Gérant, Isabelle Ziegler-Devin et Line Lapointe sont les co-auteurs de cet article. J'ai réalisé des travaux d'extraction et de détermination gravimétrique des composés structuraux et l'ensemble des analyses statistiques. Isabelle Ziegler-Devin a effectué des analyses HPAE-PAD. J'ai effectué la rédaction de cet article sous la supervision de ma directrice de recherche Line Lapointe et de ma co-directrice de recherche Dominique Gérant.

Chapitre 3. Yanwen Dong, Dominique Gérant, Sergi Munné-Bosch, Maren Müller et Line Lapointe sont les co-auteurs de cet article. J'ai réalisé l'ensemble des analyses métabolomiques et toutes les analyses statistiques menant aux résultats présentés dans ce chapitre. Sergi Munné-Bosch et Maren Müller ont effectué des mesures des phytohormones. Moustafa A. Khalf a réalisé des mesures de l'activité protéolytique. J'ai effectué la rédaction de cet article sous la supervision de ma directrice de recherche Line Lapointe et de ma co-directrice de recherche Dominique Gérant.

Introduction

Les relations source−puits

Les transferts de carbone (C) dans la plante dépendent largement des relations source−puits qui sont impliquées dans l'intégration de la production de sucres et d'acides aminés par l'assimilation photosynthétique et leurs utilisations dans la croissance, le stockage, la maintenance et la reproduction (Foyer and Paul, 2001). Généralement, «**source**» et «**puits**» sont des descriptions fonctionnelles des organes de la plante, définissant leur aptitude à fournir ou à utiliser un substrat. Les **sources de C** sont définies comme les organes exportateurs nets de C vers d'autres parties de la plante. Les **puits de C** correspondent aux organes importateurs nets de C (Farrar, 1993). Pendant la croissance, les organes sources, généralement les feuilles matures, sont photosynthétiquement actifs et exportent des photoassimilats synthétisés vers les organes puits tels que les jeunes feuilles, les racines, les organes floraux, les fruits et les organes de réserve (bulbe, corme, rhizome et tubercule) (Dickson, 1991). Certains organes peuvent alterner d'une fonction de puits à une fonction de source (Turgeon, 1989). Ainsi, les jeunes feuilles deviennent progressivement sources nettes de C lorsqu'elles se développent, et les organes de réserve ne sont pas uniquement des organes puits mais peuvent également agir comme sources de C quand les demandes en C de la plante ne peuvent être assurées par la photosynthèse.

Une définition générale de la **force d'une source** devrait prendre en compte le taux d'exportation d'une ressource particulière à partir du tissu source, qui peut-être décrit par sa taille (biomasse totale du tissu source) et son activité (taux d'absorption spécifique de la ressource) (White *et al.*, 2016). Parallèlement, la **force d'un puits** peut être caractérisée par sa capacité d'importer des photoassimilats pour des biosynthèses ou du stockage. L'activité du puits est principalement liée à l'entretien des tissus et aux dépenses énergétiques liées à l'incorporation des ressources dans les nouveaux tissus. La capacité du puits est déterminée par le nombre de cellules et leur taille (Farrar, 1993; Marcelis, 1996). Le transfert des photoassimilats depuis les organes sources vers les organes puits définit, à l'échelle de la plante entière, l'allocation des ressources. L'**allocation du C** peut varier tout au long de la saison en fonction des stades développementaux, mais de nombreux facteurs abiotiques (concentrations en dioxyde de carbone et en ozone, lumière et température) peuvent aussi

moduler le patron d'allocation en modifiant la quantité de C fixé par les plantes ou le taux de croissance des différents organes puits.

La croissance des plantes peut être influencée et limitée par la disponibilité des composés assimilés (**limitation de la source**) ou par la capacité des organes à utiliser ces assimilats (**limitation du puits**) (DeJong and Grossman, 1995; Farrar, 1996). Les plantes peuvent passer d'une croissance limitée par la source à une croissance limitée par le puits pendant des périodes spécifiques au cours de la saison de croissance ou en réponse aux changements environnementaux (Pavel and Dejong, 1993; Farrar, 1996). Par exemple, chez le blé (*Triticum aestivum* L.), la phase végétative et la floraison sont limitées par la source, alors que la phase de remplissage des grains est limitée par le puits (Waters *et al.*, 1984; Jenner *et al.*, 1991; Fischer, 2007). Une croissance limitée par le ou les puits a aussi été signalée chez certains espèces avec des organes de stockage comme l'*Helianthus tuberosus* (Schubert and Feuerle, 1997) et chez des arbres (Körner, 2003). Leuzinger *et al.* (2013) ont récemment rapporté l'impact d'une croissance limitée par le puits sur le stockage de C et la limite nordique ou altitudinale des arbres. Une limitation par les puits entraîne une augmentation de la quantité de C stocké, ce qui peut ensuite réduire le taux photosynthétique par rétro-inhibition et ainsi accélérer la sénescence foliaire (Paul and Foyer, 2001). Des études précédentes sur le *Crocus vernus* et l'*Allium tricoccum* ont trouvé que la croissance des organes pérennes souterrains (bulbe ou corme selon l'espèce) cesse avant l'initiation de la sénescence foliaire (Badri *et al.*, 2007; Lundmark *et al.*, 2009; Bernatchez and Lapointe, 2012), suggérant que la croissance de ces éphémères printanières est limitée par le puits et cette limitation se développe une fois que le puits est rempli des réserves de C (Lapointe, 2001).

Éphémères printanières

Le cycle de vie des éphémères printanières à bulbes, qui font partie des géophytes, s'organise autour de l'organe de réserve, qui joue tour à tour le rôle de source en début de saison puis le rôle de puits une fois le feuillage déployé. L'organe de réserve sert à assurer la survie des plantes, en accumulant des réserves de C et de nutriments, et la reproduction

clonale par division végétative (De Hertogh and Le Nard, 1993). Les géophytes peuvent se distinguer selon leur période de floraison: les espèces à floraison printanière telles que le *Crocus vernus*, les *Erythronium* et les *Tulipa*, les espèces à floraison estivale telles que les *Dahlia*, les *Gladiolus* et les *Lilium* et les espèces à floraison automnale comme le *Crocus sativus*, les *Colchicum* et le *Sternbergia lutea*. Notre travail portait essentiellement sur une des géophytes à floraison printanière, l'**érythrone d'Amérique** (*Erythronium americanum* Ker-Gawl). L'érythrone d'Amérique est une plante herbacée vivace à bulbe de la famille des Liliacées (Liliaceae), une des plus communes des érablières nord-américaines. Dans des conditions naturelles, une population de l'érythrone d'Amérique est composée de 3 types d'individus: *i)* plantules de première année (< 0,5 %), *ii)* individus avec une seule feuille qui peuvent se reproduire végétativement par rhizomes (> 99%), et *iii)* individus à deux feuilles qui produisent des fleurs (< 0,5 %). Ces 3 types représentent toutes les étapes du cycle de vie de cette espèce (Blodgett, 1910; Muller, 1978).

L'érythrone d'Amérique se caractérise par un cycle biologique annuel spécifique (Figure I). Une baisse de température du sol en fin d'été induit la levée de la dormance du bulbe (Risser and Cottam, 1968). On peut observer alors la mise en place d'un nouveau système racinaire, qui complète sa croissance durant l'automne. Pour sa part, le bourgeon se développe et poursuit sa croissance tout au cours de l'automne et de l'hiver. Dès la fonte des neiges, la croissance épigée débute avec le déroulement foliaire (Muller, 1978). Les différents stades phénologiques sont illustrés photographiquement dans la Figure II. Après avoir complété l'expansion foliaire, le bulbe mère s'épuise progressivement et le nouveau bulbe commence à apparaître au cœur du bulbe mère. Cette espèce présente une synthèse rapide des protéines photosynthétiques et un taux de photosynthèse assez élevé (Taylor and Pearcy, 1976), ce qui permet d'assurer une accumulation de réserves (amidon principalement) la plus rapide possible au sein du nouveau bulbe. Après avoir complété sa croissance, le bulbe entre en dormance, et l'initiation de la sénescence foliaire a lieu peu après la cessation de croissance du bulbe. En plus de sa courte durée de croissance épigée (40 à 60 jours), environ 99 % des individus de cette espèce présentent une seule feuille (qui sert de fonction de source) et un seul bulbe (donc le puits) (Blodgett, 1910), ce qui lui rend un modèle biologique très intéressant dans les études de relations source−puits.

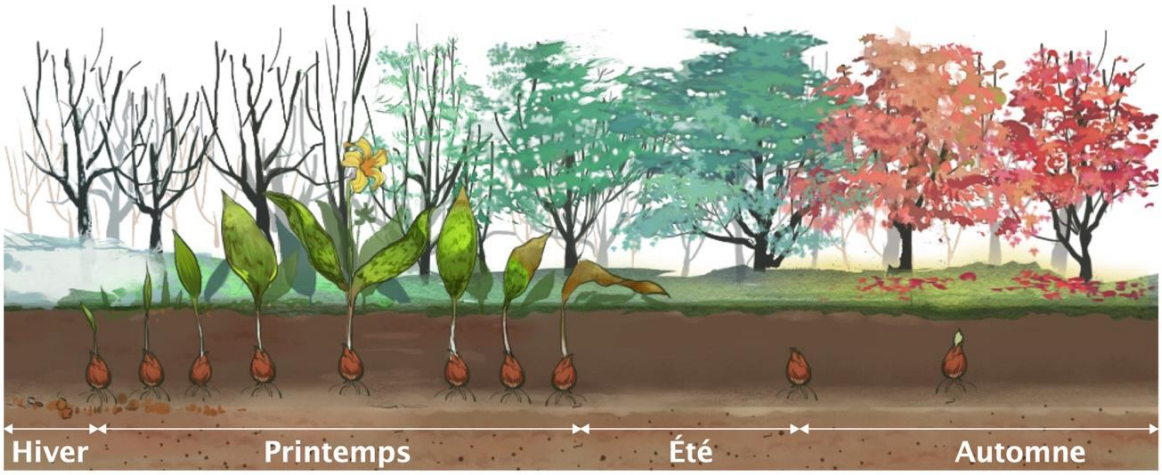


Figure I. Illustration schématique des différents stades phénologiques de l'érythrone d'Amérique en fonction de la saison. Les individus immatures possèdent une seule feuille, les individus matures en possèdent deux et fleurissent. L'échelle de temps n'est pas respectée pour des fins de clarté.

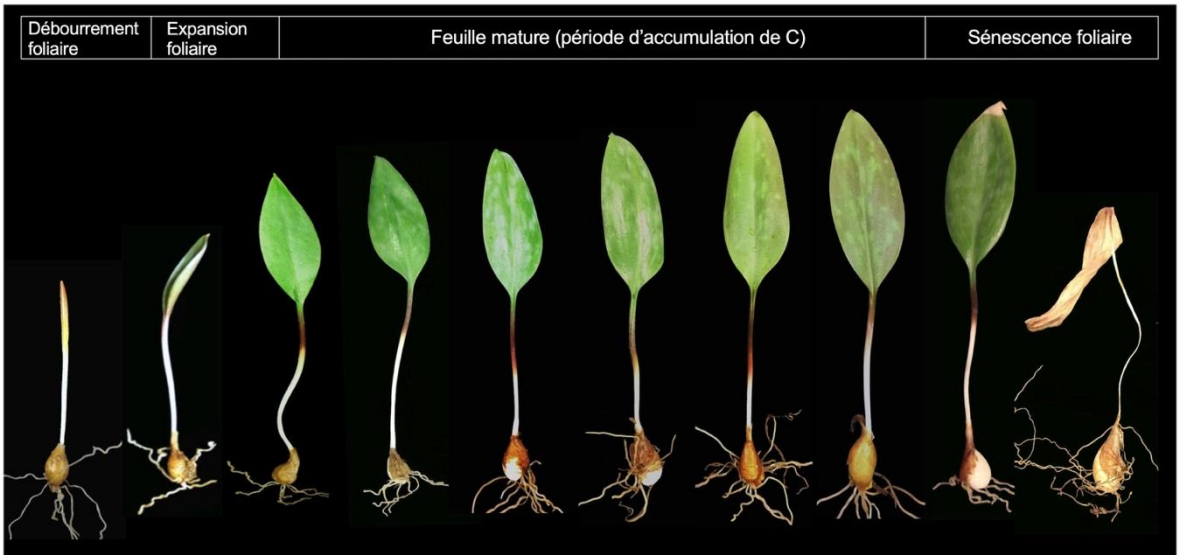


Figure II. Illustration photographique des différents stades phénologiques chez l'érythrone d'Amérique. De gauche à droite: débourrement foliaire, expansion foliaire, feuille mature (période d'accumulation de C), et sénescence foliaire.

Lapointe (2001) a proposé un modèle selon lequel l'induction de la sénescence foliaire serait due à une baisse de la demande en C des organes de réserve plutôt qu'à un effet direct de facteurs abiotiques. Des expériences effectuées par la suite sur l'érythrone d'Amérique ont supporté cette hypothèse. La longévité foliaire est limitée chez cette espèce même en conditions stables de lumière, ce qui suggère que la baisse de luminosité qui a lieu lors de la fermeture du couvert forestier est un facteur mais pas le signal principal induisant la sénescence foliaire (Lapointe and Lerat, 2006). Des expériences d'enrichissement en CO_2 ont montré que la croissance du bulbe est dépendante de la capacité de croissance cellulaire du puits plutôt que de la disponibilité de C (Gutjahr and Lapointe, 2008), soulignant donc aussi l'effet critique de la force du puits. De plus, l'érythrone d'Amérique présente une meilleure croissance lorsqu'elle pousse aux températures plus basses, avec comme résultat une durée de vie de la feuille plus longue et une période de croissance du bulbe prolongée par rapport aux températures de croissance plus élevées (Lapointe and Lerat, 2006; Gandin *et al.*, 2011). Des résultats similaires ont également été rapportés chez d'autres éphémères printanières, telles que le *Crocus vernus* et l'*Allium tricoccum* (Badri *et al.*, 2007; Lundmark *et al.*, 2009; Bernatchez and Lapointe, 2012). La limitation par les puits semble se développer au fur et à mesure que le C s'accumule dans le bulbe chez ces éphémères printanières, induisant une rétro-inhibition de la photosynthèse et finalement la sénescence foliaire. Aux basses températures, un taux d'assimilation plus faible de l'organe source coïncide avec une croissance un peu plus lente de l'organe puits par rapport aux températures plus élevées (Gandin *et al.*, 2011), ce qui entraîne un meilleur équilibre entre l'activité de la source (entrée du C par assimilation) et celle du puits (utilisation des assimilats). Ainsi la feuille dure plus longtemps et au final permet une accumulation plus importante de C dans le bulbe, avant que le déséquilibre n'induise la sénescence foliaire. Néanmoins, certaines voies métaboliques pourraient être activées afin de tenter de maintenir un puits fort dans les cas de déséquilibre source−puits. Une des possibilités serait d'augmenter l'activité métabolique du puits, e.g. son activité respiratoire. L'autre option serait d'investir le C assimilé ailleurs une fois que les cellules sont remplies d'amidon, par exemple dans des composés structuraux.

Activité respiratoire régulant la force du puits

La respiration aérobie est constituée d'une série de voies dans lesquelles les glucides sont oxydés afin de récupérer l'énergie mise en réserve au cours de la photosynthèse et de récolter des squelettes carbonés qui sont les précurseurs d'autres molécules servant à la croissance et au maintien des structures cellulaires. Les trois étapes principales de la respiration sont: (1) la glycolyse, (2) le cycle de l'acide citrique (ou cycle de Krebs) et (3) la phosphorylation oxydative qui mène à la synthèse d'ATP (Figure III). Les plantes possèdent une voie dite alternative au travers de laquelle les électrons sont transférés directement de l'ubiquinone à l'oxygène dont la réduction en eau est catalysée par l'**ubiquinol oxydase non électrogène**, ou **oxydase alternative** (**AOX**). Cette enzyme a été découverte surtout du fait de son insensibilité à certains inhibiteurs classiques du transport d'électrons (cyanure par exemple), mais elle est sensible à l'inhibition exercée par des dérivés de l'acide hydroxamique comme l'acide salicylhydroxamique (SHAM) (Møller *et al.*, 1988). Les électrons qui transitent par l'AOX contournent deux sites d'efflux de protons (complexes III et IV, Figure III). Par conséquent, le passage par l'AOX transforme l'énergie, qui normalement aurait été conservée sous forme d'ATP, en chaleur (Siedow and Berthold, 1986).

La respiration est une réaction métabolique qui dépend hautement de la température et de la concentration des substrats (Raison, 1980; Atkin *et al.*, 2005b). Le taux respiratoire peut être ajusté en vue de compenser les changements de la température de croissance, de sorte que les plantes cultivées au froid présentent des taux plus élevés à une température définie que celles cultivées dans des conditions plus chaudes (Arnone III and Körner, 1997; Atkin *et al.*, 2000b; Atkin *et al.*, 2005a). Ce processus s'appelle l'acclimatation, et elle peut entraîner généralement une réduction de la sensibilité à la température à long terme de la respiration (Larigauderie and Körner, 1995; Atkin and Tjoelker, 2003). Lorsqu'il y a homéostasie parfaite de la respiration, les taux respiratoires des plantes cultivées dans des environnements thermiques contrastés sont presque identiques (Collier, 1996; Atkin *et al.*, 2000b). Dans des conditions où la croissance est limitée par la force des puits, une stimulation du taux respiratoire des feuilles pourrait réduire les déséquilibres source–puits,

qui sont connus pour induire une sénescence précoce foliaire par rétro-inhibition de la photosynthèse (Paul and Foyer, 2001). De telles augmentations de la respiration pourraient être partiellement dues à une augmentation du flux d'électrons vers la voie respiratoire alternative non-phosphorylante (Vanlerberghe and McIntosh, 1992; González-Meler *et al.*, 1999; Florez-Sarasa *et al.*, 2007).

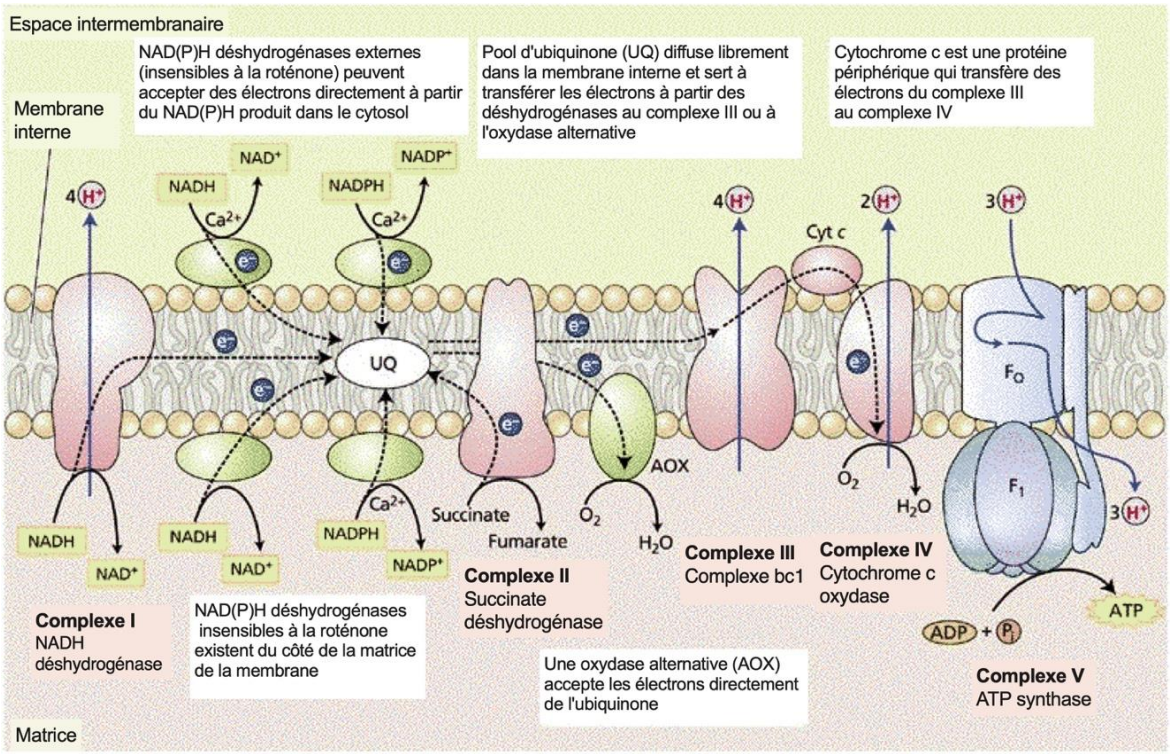


Figure III. Représentation de l'organisation de la chaîne de transport d'électrons et synthèse d'ATP au niveau de la membrane interne de la mitochondrie chez les végétaux [modifiée d'après Taiz and Zeiger (2010)].

La voie respiratoire alternative a été révélée tout d'abord en lien avec la **thermogenèse** qui, en particulier chez certaines espèces de la famille des Aracées (Araceae), provoque la volatilisation d'amines qui attirent, semble-t-il, des insectes pollinisateurs (Wagner *et al.*, 1998). Par la suite, la voie alternative a également été considérée comme un **mécanisme de protection contre les stress oxydatifs**, car elle permet de dériver le surplus d'électrons de la chaîne de transport des électrons et ainsi éviter la formation des radicaux libres (Møller, 2001). Enfin, l'un des rôles potentiels de cette voie très intéressant dans les études des relations source–puits est la consommation

des sucres en excès qui ne sont pas utilisés pour la production d'énergie, la croissance et les processus d'entretien des tissus, à savoir l'**hypothèse du trop-plein d'énergie** (« **energy overflow** ») proposée par Lambers (1982). Plusieurs études ont rapporté des résultats qui étaient compatibles avec cette hypothèse selon laquelle la voie alternative agit pour 'brûler' l'excès de sucres dans de nombreuses conditions de stress, à savoir la sécheresse et le stress lumineux (Giraud *et al.*, 2008), la faible disponibilité en azote (Noguchi and Terashima, 2006) et le stress des macronutriments (Sieger *et al.*, 2005). Gandin *et al.* (2009) ont aussi montré que la capacité de la voie alternative était fortement stimulée dans le bulbe chez l'érythrone d'Amérique dans des conditions où la photosynthèse était stimulée par des concentrations en CO_2 élevées et que la croissance était limitée par la force du puits. Il semblerait que cette voie alternative pourrait être engagée comme un moyen de réduire les déséquilibres source−puits. Bien que la caractéristique non conservatrice de l'énergie de la voie alternative devrait affecter négativement la croissance des plantes, ses effets positifs dans le maintien de l'homéostasie métabolique pourraient compenser ses influences négatives (Vanlerberghe, 2013).

Répartition du carbone dans le bulbe

Il est bien connu que les composés carbonés peuvent être principalement divisés en **glucides non-structuraux** (« non-structural carbohydrates », **NSC**) et en **glucides structuraux** (« structural carbohydrates », **SC**) (Chapin *et al.*, 1990; Hoch, 2007). L'allocation du C de la source vers le puits et la **répartition du C** (« **C partitioning** ») entre les différents pools de C non-structuraux et structuraux sont régulées par des priorités de stockage, de défense ou de croissance dans les organes puits (Chapin *et al.*, 1990). Les changements de répartition du C entre les composants de la paroi cellulaire et les composés non-structuraux pourraient aussi modifier la croissance des organes puits (Fujita *et al.*, 2004). Ainsi, la température de croissance pourrait induire une série de changements de répartition du C, ce qui pourrait expliquer la taille finale plus grande du bulbe (ou corme) des éphémères printanières aux températures élevées comme mentionné précédemment.

Parmi les NSC, l'**amidon** est la principale forme de réserve dans les organes pérennes (bulbe ou corme) de nombreuses géophytes (Miller and Langhans, 1990; Badri *et al.*, 2007; Ranwala and Miller, 2008; Gandin *et al.*, 2011). Ce glucide s'accumule en grandes quantités chez beaucoup de végétaux pendant une des phases du développement, puis est dégradé et utilisé pendant une autre phase comme la germination ou le débourrement (Chapin *et al.*, 1990; Ranwala and Miller, 2008). Les fructanes constituent un autre groupe de polysaccharides de réserve; ils sont les composés de réserve dominants chez environ 15 % des angiospermes (Hendry, 1993). Ils sont naturellement présents en solution dans les vacuoles des cellules, ce qui les distingue de l'amidon qui, lui, est séquestré dans des plastes sous forme cristalline hautement insoluble. De plus, contrairement à l'amidon, les fructanes sont solubles dans l'eau et osmotiquement actifs, et ces molécules pourraient protéger les plantes contre le froid et la sécheresse (Pollock, 1986; Hendry, 1987). Des glucomannanes non-structuraux constituent une autre forme de composés de réserve, bien que moins souvent utilisés que les fructanes, dans des tissus de stockage chez plusieurs géophytes, telles que les *Narcissus* et les *Amaryllis* (Miller, 1992). Cependant, chez les géophytes, d'autres composés carbonés peuvent également être stockés dans les organes puits au cours de la saison de croissance, bien que l'abondance de l'amidon puisse masquer leur rôle en tant que réserves de C. Lundmark *et al.* (2009) ont rapporté que l'allocation du C aux parois cellulaires augmentait en fin de saison et surtout aux températures de croissance plus chaudes chez le *Crocus vernus*, ce qui suggère un changement dans le patron de répartition des ressources une fois les cellules remplies d'amidon.

La **paroi cellulaire** forme non seulement une barrière physique pour les plantes contre les agressions biotiques et environnementales, mais elle peut également réorganiser ses composantes durant la croissance en fonction des stades de développement ou en réponse à des stress environnementaux (Cosgrove, 2005; Sarkar *et al.*, 2009). Elle est constituée d'une variété des composés différents (Figure IV), principalement des polysaccharides structuraux (cellulose, hémicelluloses et pectines), de la lignine, des protéines et d'autres composés mineurs, qui sont étroitement interconnectés. Après la cellulose, les **hémicelluloses** sont les polysaccharides les plus abondants dans la nature, et

généralement représentent 25 % de la biomasse sèche végétale totale, bien qu'elles puissent atteindre moins de 5 % chez certains fruits charnus (Thomas and Thibault, 2002), ou autant que 50 % dans certains tissus comme la paille de céréales (Hoch, 2007; Schädel *et al.*, 2010a).

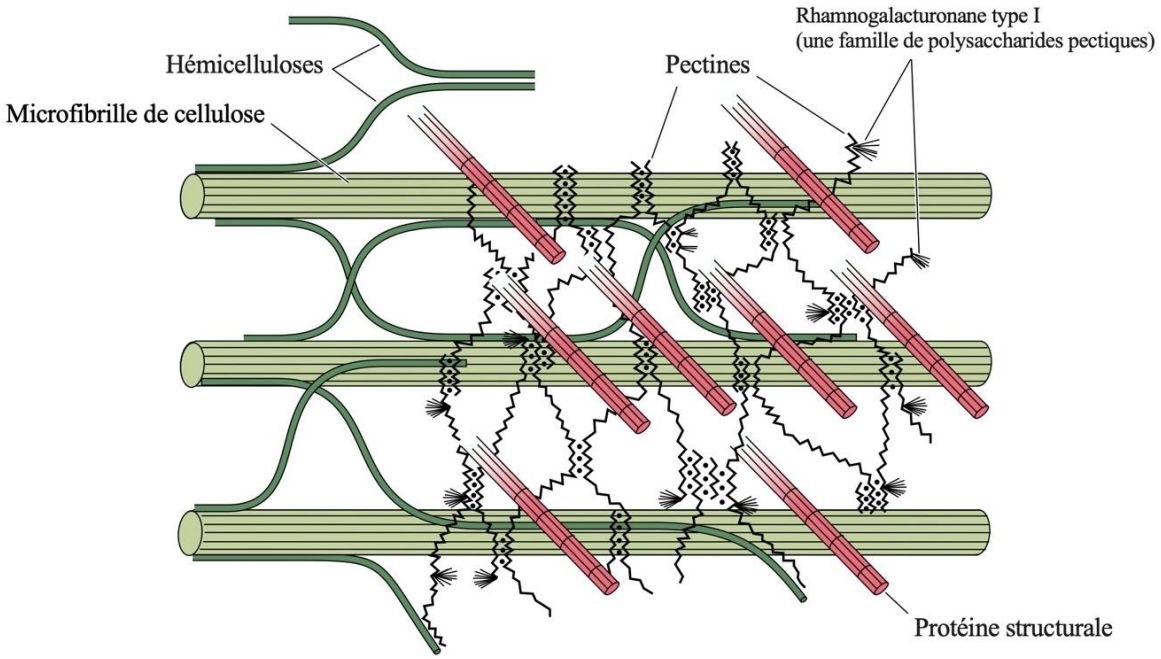


Figure IV. Représentation schématique des principaux composants structuraux de la paroi cellulaire primaire et de leur disposition probable. Les microfibrilles de cellulose sont recouvertes d'hémicelluloses, qui peuvent également réticuler les microfibrilles entre elles. Les pectines forment un gel matriciel imbriqué, pouvant éventuellement interagir avec des protéines structurales [modifiée d'après Taiz and Zeiger (2010)].

Les hémicelluloses peuvent être classées en quatre grands groupes: les xyloglucanes, les xylanes, les mannanes et les glucanes à liaisons mixtes (β-(1➔3,1➔4)-glucanes) (Ebringerova *et al.*, 2005; Scheller and Ulvskov, 2010). Les **xyloglucanes** sont les hémicelluloses les plus abondantes dans la paroi cellulaire primaire des dicotylédones. C'est la classe la mieux étudiée et ils sont constitués d'une chaîne de glucose et de courtes chaînes latérales de xylose, galactose et fucose. Ils jouent un rôle important dans l'élongation cellulaire (Pauly *et al.*, 2013). Les **xylanes**, polymères de xylose, sont essentiels dans le développement de tissus à forte teneur en parois secondaires tant chez les

espèces ligneuses que chez les espèces herbacées (Saha, 2003). Les **mannanes**, composés principalement de monomères de mannose, sont le principal groupe d'hémicelluloses dans la paroi cellulaire secondaire des gymnospermes. Ils sont particulièrement abondants dans les graines de diverses espèces, telles que le palmier dattier (*Phoenix dactylifera*), le sésame (*Sesamum indicum*) et les caféiers (*Coffea* L.) (Buckeridge *et al.*, 2000), et sont généralement considérés comme des polysaccharides de réserve dans le corme du konjac (« voodoo lily », *Amorphophallus konjac*) (Gille *et al.*, 2011). Les **glucanes à liaisons mixtes** n'ont pas été trouvés chez les plantes dicotylédones, mais sont typiques des membres de l'ordre des Poales et de quelques autres groupes, regroupant principalement des monocotylédones comme les graminées, les bromeliacées, les roseaux, les joncs et les Carex (Buckeridge *et al.*, 2004; Sørensen *et al.*, 2008).

D'après Hoch (2007), certains composants structuraux peuvent être partiellement mobiles, et les hémicelluloses présentent une mobilité relativement plus importante par rapport à la cellulose et à la lignine. L'existence d'une forme mobile de glucides dans les parois cellulaires des organes non-reproductifs apporterait un nouvel éclairage sur les relations source−puits (Chapin *et al.*, 1990; Körner, 2003). Malgré leur importance quantitative, les hémicelluloses ont été souvent négligées dans les études écophysiologiques, principalement en raison de difficultés analytiques liées à leur hétérogénéité chimique. Jusqu'à récemment, l'hétérogénéité chimique des hémicelluloses de la paroi cellulaire et le volume relativement grand des échantillons requis dans les méthodes existantes représentaient des obstacles majeurs pour des analyses interspécifiques et à grande échelle. Schädel *et al.* (2010a) ont appliqué une nouvelle méthode de micro-extraction pour analyser les hémicelluloses et le rapport entre « cellulose et lignine » et hémicelluloses dans les différents tissus chez différents groupes fonctionnels de plantes. Dans leur étude, l'analyse des fibres de Van Soest (Van Soest, 1963; Van Soest and Wine, 1967) a été modifiée pour permettre les mesures simultanées quantitatives et qualitatives des hémicelluloses sur de petits volumes d'échantillon. Dans de nombreuses études, il a été suggéré que les hémicelluloses sont aussi importantes que l'amidon dans le rôle de C de stockage dans les graines (Reid, 1985; Scheller and Ulvskov, 2010). Les concentrations élevées d'hémicelluloses dans la plupart des tissus végétaux ont été qualifiées comme des

réserves importantes potentielles de C pendant la débourrement où l'apport de C par l'assimilation photosynthétique est encore limité (Schädel *et al.*, 2009); elles agissent donc probablement en tant que pool de stockage de C supplémentaire (Hoch, 2007). En tenant compte du double rôle des hémicelluloses (polysaccharides de la paroi cellulaire et forme potentielle de réserve de C), il semble que cette fraction structurale pourrait être impliquée dans les changements de répartition du C en réponse aux variations de l'activité de la source ou du puits et ainsi pourrait influencer le bilan C total des plantes. Sachant que les cellules du parenchyme dans les bulbes ou cormes ont des parois cellulaires très minces qui s'effondrent au printemps suivant lorsque l'organe est vidé, il est donc très probable que la répartition du C aux composantes de la paroi cellulaire en fin de saison constitue une forme de réserve sous forme d'hémicelluloses plutôt qu'un investissement supplémentaire en cellulose ou lignine.

Sénescence foliaire et facteurs endogènes

La dernière étape du processus de développement d'un organe est la **sénescence**. C'est un processus génétiquement contrôlé, résultant de la succession d'évènements biochimiques et physiologiques coordonnés au niveau cellulaire et tissulaire qui conduisent à la mort de la plante ou d'un organe de la plante (Gan and Amasino, 1997; Noodén *et al.*, 1997; Thomas, 2013). La sénescence peut avoir lieu dans différents organes: on parle ainsi de sénescence foliaire, florale, ou associée aux fruits (Feller and Fischer, 1994). Ce phénomène, bien que présent chez tous les végétaux, caractérise certains groupes fonctionnels telles les plantes annuelles où la sénescence de l'ensemble de la plante a lieu en fin de saison, ou alors les espèces dites éphémères, dont la sénescence du feuillage a lieu tôt après son apparition. Au cours de la sénescence, les feuilles s'engagent dans un processus de dégradation massive de leurs macromolécules (lipides, glucides, acides nucléiques et protéines). Les éléments issus de ces dégradations (C, N, P…) sont ensuite redistribués vers les différents puits (Gan and Amasino, 1997): organes en croissance tels que les jeunes feuilles et les graines, ou organes de réserve. Munné-Bosch (2007) a déterminé trois phases de la **sénescence foliaire** chez les plantes pérennes: *i)* la phase initiale caractérisée par des cascades de signalisation précoces qui entraînent des changements dans l'expression des gènes et déclenchent l'induction du

processus de sénescence, *ii)* la phase de réorganisation avec tous les changements métaboliques et les modifications structurelles cellulaires qui sont liées à la remobilisation des éléments nutritifs, et enfin *iii)* la dernière étape irréversible de la sénescence, la phase terminale, qui est le résultat de l'accumulation de facteurs qui conduisent à une perte totale de l'intégrité cellulaire et finalement à la mort des cellules. Le processus de sénescence, y compris le taux de sénescence, est fortement influencé par divers facteurs environnementaux, tels que des températures élevées ou basses, la sécheresse, l'ozone, la déficience en nutriments, les infections par des pathogènes, l'ombrage, etc., et des facteurs internes qui comprennent les différentes phytohormones et voies métaboliques (Lim *et al.*, 2007). Il est évident que de multiples voies qui répondent à divers facteurs internes et externes doivent être reliées entre elles pour former un réseau complexe qui régule la sénescence.

Les principales **phytohormones** associées à la sénescence sont l'acide abscissique (ABA), l'acide jasmonique (JA), l'éthylène et l'acide salicylique (SA), qui sont également largement impliqués dans les réponses aux divers stress biotiques et abiotiques (Lim *et al.*, 2007). Cependant, d'autres hormones sont également impliquées dans le contrôle de la sénescence. Les cytokinines (CKs), telles que la *trans*-zéatine (Z) et l'isopentényladénine (IPA), peuvent réguler la progression de la sénescence foliaire (Roitsch and Ehneß, 2000). L'auxine (IAA) et les gibbérellines (GA) sont capables de retarder l'initiation de la sénescence foliaire (Arrom and Munné-Bosch, 2012; Mueller-Roeber and Balazadeh, 2014). Outre ses effets bien connus sur la croissance et le mûrissement des fruits, l'éthylène est aussi capable de moduler la progression de la sénescence foliaire (Lim *et al.*, 2007; Thomas, 2013). D'après les travaux sur ces phytohormones, il apparaît très clairement qu'elles ont une action centrale sur la régulation du déclenchement et de la progression de la sénescence (Arrom and Munné-Bosch, 2012). Les voies de signalisation hormonales influencent de nombreuses réponses développementales et environnementales chez les plantes, et chaque hormone végétale affecte divers événements développementaux d'une manière complexe. Ceci entraîne des difficultés dans la détermination des rôles des voies hormonales dans la sénescence foliaire (Lim *et al.*, 2007).

Par ailleurs, d'autres **métabolites** jouent également un rôle clé dans la régulation de la croissance des plantes. Par exemple, les sucres sont les régulateurs du développement des plantes les plus fréquemment signalés (Diaz *et al.*, 2005; Wingler *et al.*, 2006; Eveland and Jackson, 2012; Lastdrager *et al.*, 2014), puisqu'ils agissent à la fois comme substrats directs ou intermédiaires métaboliques et comme molécules de signalisation efficaces (Meyer *et al.*, 2007; Smeekens *et al.*, 2010). Les sucres peuvent aussi interagir avec les phytohormones pour moduler la croissance des plantes, telle que celle des tubercules (Jackson, 1999; Gibson, 2004). Il existe de plus en plus de preuves démontrant que les sucres exercent une influence régulatrice sur la sénescence, mais leurs effets sur la sénescence foliaire restent controversés et diffèrent selon les espèces (Rolland *et al.*, 2006; Wingler and Roitsch, 2008; Thomas, 2013). Plusieurs études ont rapporté que l'expression des gènes associés à la sénescence (SAGs) peut être induite non seulement par une augmentation du niveau des sucres, mais également par une diminution importante de la teneur en sucres (Yoshida, 2003; Contento *et al.*, 2004; Wingler *et al.*, 2006; van Doorn, 2008; Li *et al.*, 2017). En plus des sucres, d'autres métabolites présentent des effets sur la sénescence. Les acides aminés jouent un rôle fondamental dans la croissance des plantes, et les variations de leur contenu sont fortement liées à des stades spécifiques (Jeong *et al.*, 2004; Diaz *et al.*, 2005). Par exemple, il a été démontré que l'accumulation de la proline pouvait agir en tant qu'indicateur de la sénescence foliaire chez les cultivars de riz (*Oryza sativa*) (Wang *et al.*, 1982; Mondal *et al.*, 1985). L'abondance de l'acide γ-aminobutyrique, ou GABA, s'accroit avec la sénescence foliaire chez les plants de tabac (*Nicotiana tabacum* L.) et l'Arabidopsis (Masclaux *et al.*, 2000; Diaz *et al.*, 2005). L'acide ascorbique, un acide organique ayant des propriétés antioxydantes, joue aussi un rôle important dans la régulation du processus de sénescence (Barth *et al.*, 2006). En outre, une stimulation du catabolisme des acides gras a été observée lors de la sénescence foliaire de tabac (Li *et al.*, 2017).

Au niveau macroscopique, la sénescence foliaire se caractérise par un jaunissement au niveau de la feuille, qui résulte de la dégradation des chlorophylles et aboutit au dessèchement complet et finalement à l'abscission foliaire. Les cellules du mésophylle sont touchées en premier par la sénescence, alors que les tissus vasculaires sont touchés plus

tardivement (Gan and Amasino, 1997). En effet, les tissus proches des nervures seraient préservés dans le but d'assurer le transport des éléments issus de la dégradation. Ces éléments sont ensuite redistribués vers les organes puits (organes en croissance ou organes de réserve). Étant donné que le nitrate et les acides aminés sont peu accumulés dans les feuilles, les protéines constituent la principale source d'azote au cours du processus de remobilisation liée à la sénescence. Au cours de la sénescence foliaire, les protéines chloroplastiques sont rapidement et fortement hydrolysées. Des études chez le colza (*Brassica napus* L.) ont montré que la quantité de protéines solubles était un meilleur indicateur de la sénescence foliaire que la teneur en chlorophylle (Gombert *et al.*, 2006). Il semble que certains métabolites pourraient mieux caractériser la sénescence foliaire que les chlorophylles.

Dans la majorité des cas, les phytohormones et autres métabolites impliqués dans l'induction de la sénescence ont été étudiés principalement de manière individuelle. La voie de signalisation qui induit la sénescence foliaire n'a été que partiellement décrite, et elle semble varier en fonction de la cause de la sénescence. Les nouvelles technologies telles que la **métabolomique** nous permettent d'étudier le rôle potentiel d'un pool beaucoup plus important de métabolites dans l'induction de la sénescence foliaire (Roessner *et al.*, 2000; Diaz *et al.*, 2005; Wingler and Roitsch, 2008; Moschen *et al.*, 2016). De plus, une nouvelle méthode sensible et rapide proposée par Müller and Munné-Bosch (2011) permet d'analyser les changements dynamiques dans les concentrations endogènes de plusieurs phytohormones (**profil hormonal**) en réponse à des stress biotiques et abiotiques à l'aide de la spectrométrie de masse par chromatographie en phase liquide ultra-performante (UPLC/ESI-MS/MS), ce qui donc peut s'avérer très utile pour compléter les études métabolomiques.

Problématique

Facteurs régulant la force du puits dans les situations de déséquilibres source–puits

Nous avons voulu, dans cette étude, approfondir notre compréhension des facteurs intrinsèques qui influencent la croissance des organes de réserve chez les géophytes et qui expliquent les fortes croissances observées aux faibles températures chez plusieurs de ces espèces. Les travaux précédents suggèrent que cette croissance accrue aux basses températures est liée à un meilleur équilibre entre les activités des organes sources et puits (Lapointe, 2001; Gandin *et al.*, 2011).

Dans un premier temps, nous voulions vérifier si une respiration accrue aux températures basses, soit au niveau de la feuille ou du bulbe, pourrait permettre de maintenir la force du puits de C plus longtemps, en évitant une trop forte accumulation de sucres dans le bulbe. Nous souhaitions aussi savoir si la respiration de la feuille ou du bulbe pourrait être modulée dans le temps à mesure que la limitation du puits se développe. Pour ce faire, nous avons quantifié l'importance de la respiration aérobie et de la voie alternative, à la fois au niveau de la feuille et au niveau du bulbe, en fonction de la température de croissance (jour/nuit 8/6 °C, 12/8 °C et 18/14 °C) tout au long de la saison de croissance. Nous avons aussi évalué en même temps la contribution relative de la voie alternative et de la voie cytochromique. Les taux respiratoires ont été mesurés à la fois aux températures de croissance testées (8 °C, 12 °C et 18 °C) et à une température commune (12 °C) afin de distinguer l'effet direct de la température mesurée du processus d'acclimatation. Nous avons émis l'hypothèse que la respiration totale et la respiration alternative pourraient être stimulées aux températures de croissance basses au niveau de la feuille et du bulbe chez l'érythrone d'Amérique afin de mieux ajuster le C nécessaire à la croissance avec le C disponible par l'assimilation.

Dans un deuxième temps, nous voulions vérifier si les plantes pouvaient ajuster leur répartition du C dans les différents composés (structuraux *vs.* non-structuraux) au fur et à mesure que la limitation du puits se développe. Nous souhaitions savoir si cette répartition

du C dans les composantes de la paroi cellulaire en fin de saison de croissance serait plus importante chez les plantes poussant à des températures plus élevées par rapport à celles ayant poussé à des températures faibles, comme suggéré par Lundmark *et al.* (2009). Nous avons émis l'hypothèse que la répartition du C dans des pools de composés structuraux pourrait être modifiée en réponse aux traitements de température. Une fois que les cellules sont remplies d'amidon, les plantes cultivées à des températures plus élevées pourrait investir le C assimilé ailleurs et très probablement davantage dans certains constituants de la paroi cellulaire comme les hémicelluloses, afin de maintenir la force de leur puits. Pour ce faire, nous avons analysé les modifications qualitatives et quantitatives des composés carbonés non-structuraux et structuraux du bulbe chez les plantes cultivées à trois températures de croissance (jour/nuit 8/6 °C, 12/8 °C et 18/14 °C) à la fin de la saison de croissance. Nous avons développé une méthode combinant les protocoles de Ranwala and Miller (2008) pour analyser les concentrations et la composition des composés non-structuraux par la chromatographie ionique couplée à l'ampérométrie pulsée [High-Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD)], et de Schädel *et al.* (2010a) pour analyser la composition des pools structuraux par une méthode de micro-extraction. La teneur en C de la matière organique initiale et de chaque monosaccharide ou polysaccharide purifié a aussi été mesurée ou calculée, ce qui nous a permis de déterminer l'effet des trois températures de croissance sur la répartition du C dans le bulbe de l'érythrone d'Amérique à la fin de la saison de croissance.

Caractérisation de la sénescence foliaire

Une diminution de l'activité des puits (organes de réserve) entraîne souvent la sénescence foliaire. Même si le phénomène a été rapporté chez plusieurs espèces, les voies signalétiques n'ont pas encore été complètement élucidées. Notre travail visait à caractériser les profils phytohormonaux et métabolomiques associés à l'induction de la sénescence foliaire et à identifier les voies signalétiques qui pourraient établir le lien entre la diminution de la force du puits et l'induction de la sénescence foliaire chez l'érythrone d'Amérique. Nous voulions mettre en lumière un profil phytohormonal et métabolomique général associé à l'induction de la sénescence foliaire distinct des profils spécifiques aux

différentes températures de croissance. Les relations source–puits ont été modulées en utilisant différents régimes de température de croissance (jour/nuit 8/6 °C, 12/8 °C et 18/14 °C). Les analyses ont été effectuées tout au long de la saison de croissance, tant au niveau de la feuille que du bulbe.

Dans un premier temps, nous avons caractérisé le rôle joué par certaines phytohormones dans la voie signalétique menant à l'induction de la sénescence foliaire. Des analyses métabolomiques ont également été réalisées afin de déterminer si certains métabolites connus comme facteurs d'induction de la sénescence sont présents chez cette espèce. Nous supposons que certains sucres spécifiques pouvaient s'accumuler ou diminuer fortement quelques jours avant le premier signe visuel de la sénescence foliaire, induisant la synthèse de phytohormones telles que l'éthylène, l'ABA, le SA ou le JA, puis la sénescence foliaire. Dans un deuxième temps, nous avons quantifié les acides aminés totaux, les sucres solubles ainsi que les concentrations en protéines solubles totales afin de compléter les données métabolomiques qui sont semi-quantitatives. Les activités des protéases foliaires ont également été mesurées pour confirmer que le signal visuel de la sénescence foliaire correspondait bien au moment réel de l'induction de la remobilisation de l'azote et donc de la sénescence foliaire. S'il existe effectivement des facteurs de signalisation de la sénescence foliaire, nous supposions qu'ils devaient se comporter de la même manière au niveau de la feuille et du bulbe afin de synchroniser l'activité de la source et celle du puits.

La croissance de l'érythrone d'Amérique devient assez rapidement limitée par l'activité du puits, ce qui semble causer la sénescence précoce de toute la partie aérienne. Les analyses ciblées sur les facteurs régulant la force du puits et les potentiels indicateurs de la sénescence foliaire nous ont permis de mieux caractériser le lien entre l'activité du puits et l'induction de la sénescence foliaire chez cette géophyte printanière. Une meilleure compréhension des différentes stratégies modulant la force du puits chez cette espèce pourrait nous aider à mieux cerner leurs réponses et adaptations physiologiques à des températures de croissance changeantes.

Chapitre 1. Thermal acclimation of leaf respiration as a way to reduce source–sink imbalance at low temperatures in *Erythronium americanum*, a spring ephemeral

Yanwen Dong[1,2], Dominique Gérant[2] and Line Lapointe[1]

[1] Département de Biologie and Centre d'Étude de la Forêt, Université Laval, Québec, QC, Canada

[2] Unité Mixte de Recherches 1434 SILVA, Université de Lorraine/INRA/AgroParisTech, Faculté des Sciences et Technologies, 54506 Vandœuvre-lès-Nancy cedex, France

Botany **96**: 97–109 (2018) dx.doi.org/10.1139/cjb-2017-0168

Résumé

Plusieurs géophytes printanières croissent mieux à des températures plus fraîches que plus chaudes. Des études antérieures ont suggéré que le déséquilibre entre l'activité de la source et celle du puits était moins grand à basse température, ce qui retarde la sénescence foliaire et conduit à une accumulation plus grande de biomasse dans l'organe pérenne. Nous avons émis l'hypothèse que la respiration à l'obscurité s'acclimate à la température tant au niveau de la feuille que du bulbe, principalement par la voie alternative, comme moyen de réduire le débalancement source−puits. Des plants d'*Erythronium americanum* Ker-Gawl. ont été cultivés suivant trois régimes de températures: 8/6 °C, 12/8 °C and 18/14 °C (jour/nuit). Les taux respiratoires des plantes ont été mesurés à la température de croissance et à une température commune afin de déterminer si les différences étaient dues à des effets directs de la température sur les taux respiratoires ou à l'acclimatation. La respiration des feuilles à l'obscurité était homéostatique, ce qui, avec une assimilation plus faible à une température de croissance faible, réduisait fort probablement la quantité de C disponible pour la translocation vers le bulbe. Aucune acclimatation thermique n'était manifeste au niveau du puits. Cependant, la respiration totale du bulbe variait en fonction du temps, ce qui suggère que la respiration du bulbe est possiblement stimulée au fur et à mesure qu'une limitation par les puits se développe. En conclusion, l'acclimatation de la respiration au niveau de la feuille pourrait partiellement expliquer le meilleur équilibre entre l'activité de la source et celle du puits chez les plantes cultivées à basse température, alors que la respiration du bulbe répond au débalancement source−puits.

Mots-clés: relation source−puits, acclimatation thermique, limitation par les puits, *Erythronium americanum*

Abstract

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

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

1.1 Introduction

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

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

carbohydrate accumulation could still take place under low temperatures, which could increase the leaf R_T / A quotient (Atkin *et al.*, 2005a; Campbell *et al.*, 2007).

Another condition where carbohydrates could accumulate within the leaves is under conditions of sink-limited growth (Basu *et al.*, 1999; Hoch *et al.*, 2002). Under such conditions, stimulating leaf R_T could reduce source–sink imbalances, which are known to induce early leaf senescence through feedback inhibition (Gandin *et al.*, 2009). Such increases in respiration could be due, in part, to increased electron flow to the alternative respiratory pathway (Vanlerberghe and McIntosh, 1992; Gonzàlez-Meler *et al.*, 1999; Florez-Sarasa *et al.*, 2007). One of the potential roles for the alternative respiratory pathway is the consumption of excess carbohydrates that are not used for energy production, growth and maintenance processes in tissues, namely the "energy overflow" hypothesis proposed by Lambers (1982). Indeed, several studies have reported results that were consistent with the hypothesis that the alternative pathway acts to burn excess carbohydrate under many stress conditions, namely drought and light stress (Giraud *et al.*, 2008), low N availability (Noguchi and Terashima, 2006), and macronutrient stress (Sieger *et al.*, 2005). Gandin *et al.* (2009) have previously shown that the capacity of the alternative pathway (R_{alt}) was strongly stimulated in the bulb of spring ephemerals under sink-limited conditions that were caused by plant exposure to elevated CO_2 concentrations. Although the non-energy conserving nature of the alternative pathway would be expected to negatively affect plant growth, its positive effects in the maintenance of metabolic and signalling homeostasis might more than offset its negative effects (Vanlerberghe, 2013).

In spring ephemerals, the growth of the perennial organ (bulb or corm, according to the species) was shown to be higher at low temperatures compared with that recorded at warmer temperatures (Lapointe and Lerat, 2006; Badri *et al.*, 2007; Lundmark *et al.*, 2009; Gandin *et al.*, 2011; Bernatchez and Lapointe, 2012). As the new bulb/corm accumulates carbohydrates, sink limitation builds up, inducing feedback inhibition of photosynthesis and eventually leaf senescence (Badri *et al.*, 2007; Gandin *et al.*, 2011). It has been suggested that an enhanced bulb/corm growth at low temperature is due to the capacity of the plant to maintain a better equilibrium between source and sink activities over time

(Gandin *et al.*, 2011). Therefore, sink limitation and consequent feedback inhibition on photosynthetic activity is postponed at lower temperatures, resulting in a longer leaf life duration and a longer period of bulb/corm growth. However, a number of questions remain: Could this equilibrium be linked not only to reduced assimilation, but also to increased respiration at low growth temperature? Could R_{alt} be involved in the modulation of leaf or bulb R_T as a response to temperature? Could respiration be modulated both at the leaf and at the bulb levels to more efficiently balance the amount of C that is translocated from leaf to sink and the amount of C that is used at the sink level? Finally, could leaf or bulb R_T be modulated through time as sink limitation builds up?

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

1.2 Material and Methods

1.2.1 Plant Material and Experimental Design

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

1.2.2 Plant Phenological Stages

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

complete leaf senescence; bulb enters into dormancy). In the present study, the different variables were measured at specific sampling stages (identified as T1 to T5) during the season, which covered the different phenological stages of both leaf and bulb (Fig. 1-1).

1.2.3 Leaf Assimilation Measurements

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

1.2.4 Leaf and Bulb Respiration Measurements

One hundred milligrams of fresh leaf discs (6 mm in diameter) and bulb pieces (4 mm × 4 mm ×1 mm) were sampled from five plants per growth chamber per sampling stage, rinsed and vacuum infiltrated in a syringe with reaction medium. The medium contained 100 mmol.L^{-1} mannitol, 10 mmol.L^{-1} HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 10 mmol.L^{-1} MES (2-(N-morpholino)ethanesulfonic acid, pH 6.6) and 0.2 mmol.L^{-1} $CaCl_2$, according to the method described by Jolivet *et al.* (1990). For the samples that were harvested at 50 % of leaf senescence (T5), a 50:50 mixture of green and yellow leaf sections were used. The fragments were transferred to a dissolved O_2 electrode incubation chamber (Rank Brothers Ltd., Cambridge, UK). O_2 uptake was measured with this Clark-type polarographic electrode in 4 mL of air-saturated reaction medium under the day-growth temperatures (i.e., 8 ℃, 12 ℃ and 18 ℃, respectively), and at a common temperature of 12 ℃. Aqueous KCN (1 mmol.L^{-1}) and salicylhydroxamic acid (SHAM, 10 mmol.L^{-1}), which was dissolved in methoxy-ethanol, were used as inhibitors of the cytochrome pathway and of the alternative pathway, respectively. The O_2 electrode chamber was covered with aluminium foil during leaf measurement, to ensure that leaf dark respiratory rates were being measured.

For both organs, total respiratory rate (R_T) was measured at first without any inhibitors. The capacity of the alternative pathway (R_{alt}) was determined using the equation: $R_{alt} = R_{T+KCN} - R_{res}$, where R_{T+KCN} is the respiratory rate that was measured after the addition of the inhibitor KCN, and R_{res} (residual respiratory rate) was measured by adding the second inhibitor, SHAM. The capacity of the cytochrome pathway (R_{cyt}) was determined using the equation: $R_{cyt} = R_{T+SHAM} - R_{res}$, where R_{T+SHAM} is the respiratory rate that was measured when the inhibitor SHAM was added first.

All leaf gas exchange data (assimilation and respiration measurements) were converted to the same units (µmol O_2. min^{-1}. g dry $mass^{-1}$; dry mass, DM). Given that the number of replicates in the assimilation measurements (n = 5) and the respiration measurements (n = 2 to 4) were unequal, and done on different samples, we used a permutation approach to calculate between 10 and 20 different quotients of leaf dark respiration to net assimilation (leaf R_T / A) per growth temperature and phenological stage. All these estimated quotients were used as replicates in the statistical analysis. We considered that there were only 5 replicates when calculating the standard error of the mean in order to avoid under-estimating it.

1.2.5 Non-Structural Carbohydrate Concentrations

For each treatment and at each sampling stage, five plants were harvested 3 hours after the beginning of the light period. Leaves and bulbs were separated and flash-frozen in liquid nitrogen. Plant material was then freeze-dried, weighed, and ground into fine powder in a ball mill (Qiagen Inc., Toronto, Canada). Soluble sugars were analysed from both leaf and bulb samples, whereas starch was only analysed from the bulb samples, given that leaves in this species do not contain significant quantities of starch (Gandin *et al.*, 2009). Fifty milligrams of freeze-dried ground material was macerated for 20 minutes in a methanol-chloroform-water solution (MCW 12:5:3, v/v) at 65 °C. After homogenisation using a Polytron (Kinematica, Lucerne, Switzerland), the mixture was centrifuged at 2 200g for 10 minutes at 4 °C. The supernatant was then harvested and stored temporarily on ice. To complete the extraction of sugars from the remaining pellet, homogenisation and

centrifugation were repeated, and the second supernatant was added to the first one. Total soluble sugars were analysed by reaction of 100 µL of the supernatant with 1.5 mL of freshly prepared anthrone solution (Hansen and Møller, 1975) in warm water (60 °C) for 20 minutes. After cooling, the absorbance was determined at 620 nm with a spectrophotometer (Beckman DU640, Beckman Coulter, USA). Glucose was used as the standard.

The pellet containing starch was gelatinised in boiling water for 90 minutes, then hydrolysed at 55 °C for 60 minutes in the presence of amyloglucosidase (Sigma-Aldrich, St. Louis, Missouri, USA). After cooling and centrifugation (2 200g for 5 minutes), starch concentration was determined colorimetrically at 415 nm on a glucose-equivalent basis using p-hydroxybenzoic acid hydrazide (Blakeney and Mutton, 1980).

1.2.6 Statistical Analysis

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

1.3 Results

1.3.1 Plant Phenology

Growth duration of *E. americanum* decreased as growth temperature increased, lasting 73 days, 56 days, and 43 days for plants grown under the 8/6 °C, 12/8 °C and 18/14 °C regimes, respectively (Fig. 1-1). Similar responses were exhibited for the individual phenological stages (periods I to V). Leaf expansion (period I) lasted 9 days, 7 days and 5 days for plants grown at 8/6 °C, 12/8 °C, and 18/14 °C, respectively. New bulb growth started within the core of the old bulb at T2 (period III of the green-leaf period), which

occurred at days 19, 15 and 11 for plants grown at 8/6 ℃, 12/8 ℃, and 18/14 ℃, respectively (Fig. 1-1). Initiation of leaf senescence (T4), which also corresponds to the termination of new bulb growth, was recorded at days 47, 34 and 24 for plants grown at 8/6 ℃, 12/8 ℃, and 18/14 ℃, respectively. Leaf senescence (periods IV + V) also lasted longer under the coolest growth temperature regime (Fig. 1-1).

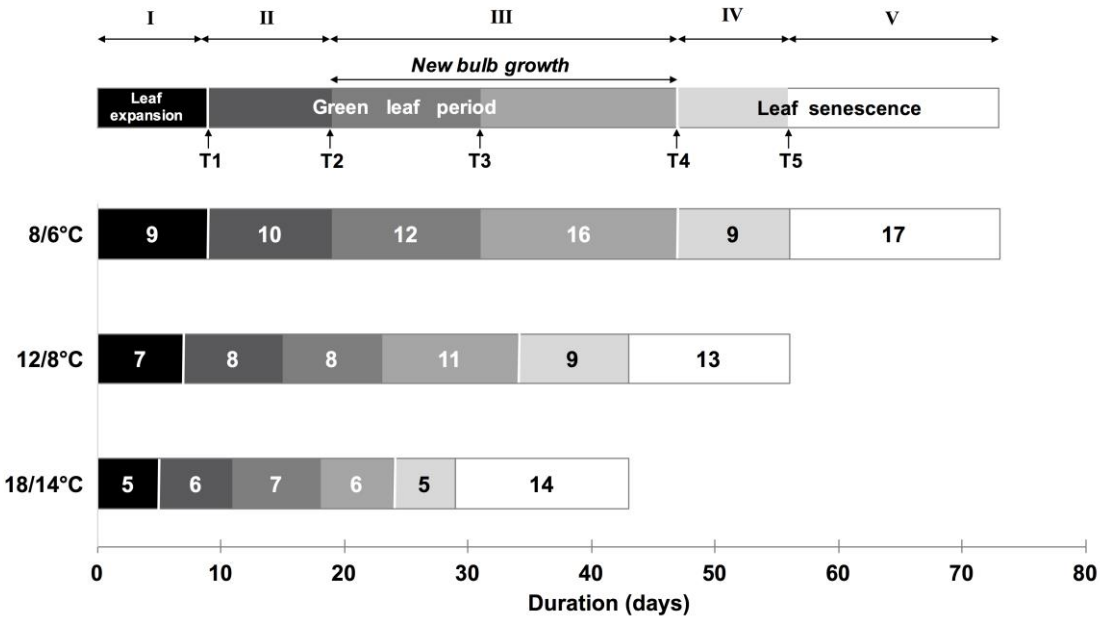


Figure 1-1. Representative illustration of plant phenology of *E. americanum* grown at day/night temperatures of 8/6 ℃, 12/8 ℃, and 18/14 ℃. Shown from left to right: duration of leaf expansion (period I), duration of green leaf (period II and III), and leaf senescence period (period IV and V). Arrows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

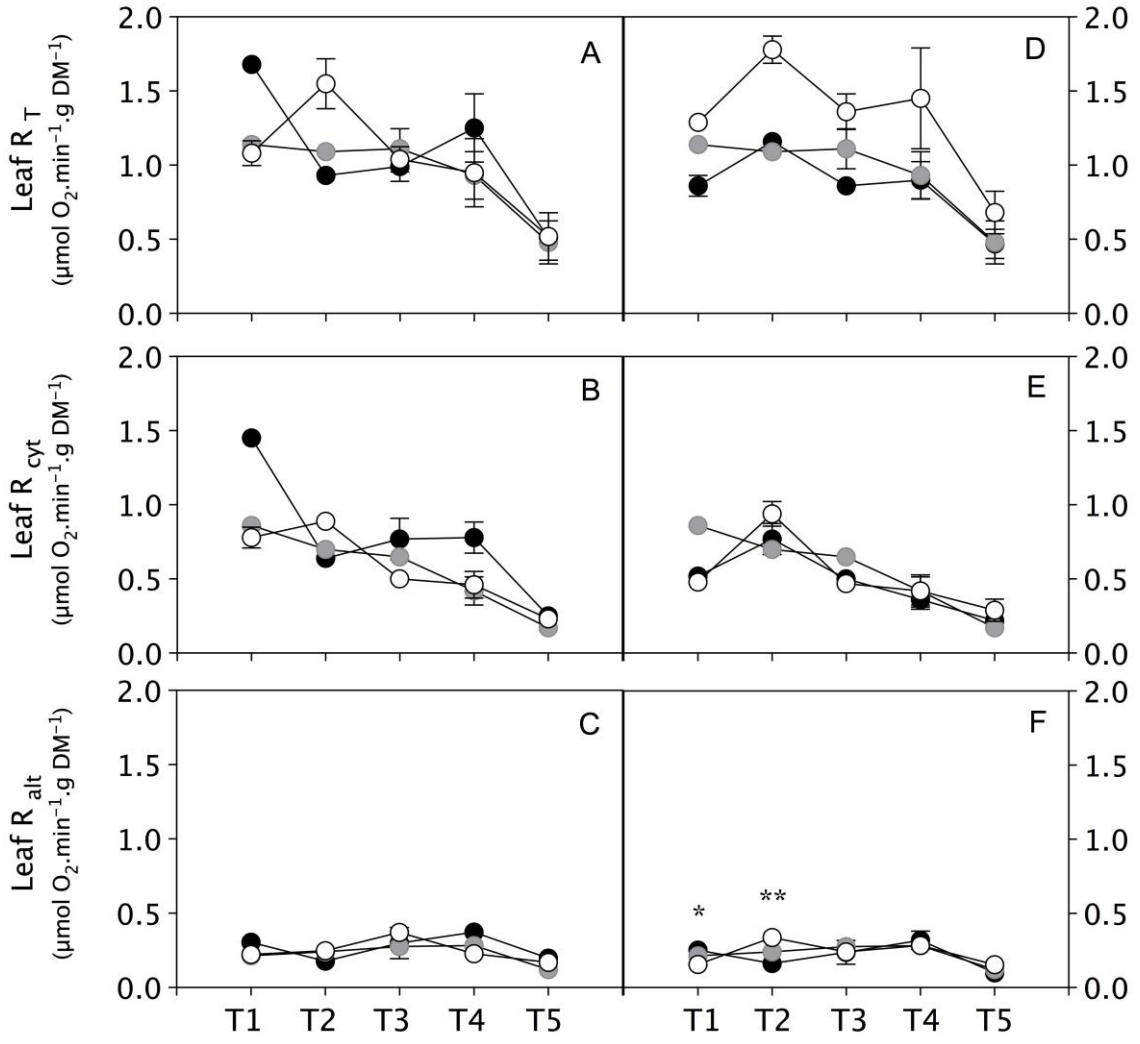


Figure 1-2. Total dark respiratory rate (leaf R_T, µmol O_2. min^{-1}. g DM^{-1}, A and D), capacity of the cytochrome pathway (leaf R_{cyt}, µmol O_2. min^{-1}. g DM^{-1}, B and E) and capacity of the alternative pathway (leaf R_{alt}, µmol O_2. min^{-1}. g DM^{-1}, C and F) in the leaves of plants grown at 8/6 ℃ (white), 12/8 ℃ (grey), and 18/14 ℃ (black) during the green leaf period and leaf senescence (T1 to T5), measured at growth temperature (A – C) and a common temperature of 12 ℃ (D – F). Values for the means ± standard error of the mean (SEM) are presented (n = 2 to 4). * and ** denote that the respiratory rates differed for at least two out of the three growth temperatures at $P < 0.05$ and < 0.01, respectively, within each stage when Temperature × Stage interaction effect was significant. See Table 1-1 for results of multiple test comparisons.

1.3.2 Leaf Dark Respiratory Rates

Leaf R_T was fairly constant throughout the growing season, except in senescing leaves (T5) where it decreased (Fig. 1-2A and Table 1-1). R_{cyt} was highest at the beginning of the growth season when the leaf was expanding, and gradually decreased with time until senescence (Fig. 1-2B and Table 1-1). R_{alt} remained fairly constant over most of the season (Fig. 1-2C and Table 1-1), except during leaf senescence when it declined. Plants grown at the three different growth temperatures exhibited similar values for R_T and R_{alt} when measured at their respective growth temperatures (Figs. 1-2A and 1-2C; Table 1-1), whereas R_{cyt} was generally higher in plants grown at the highest growth temperature (Fig. 1-2B and Table 1-1).

When respiratory rates were measured at a common temperature (12 ℃), R_T was stimulated in cold-grown plants, but not through an increase in the capacity of either the cytochrome or the alternative respiratory pathway (Figs. 1-2D and 2E; Table 1-1). An enhanced effect of low growth temperature on R_{alt} was observed only at T2 (Fig. 1-2F).

1.3.3 Leaf A and R_T / A Quotient

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

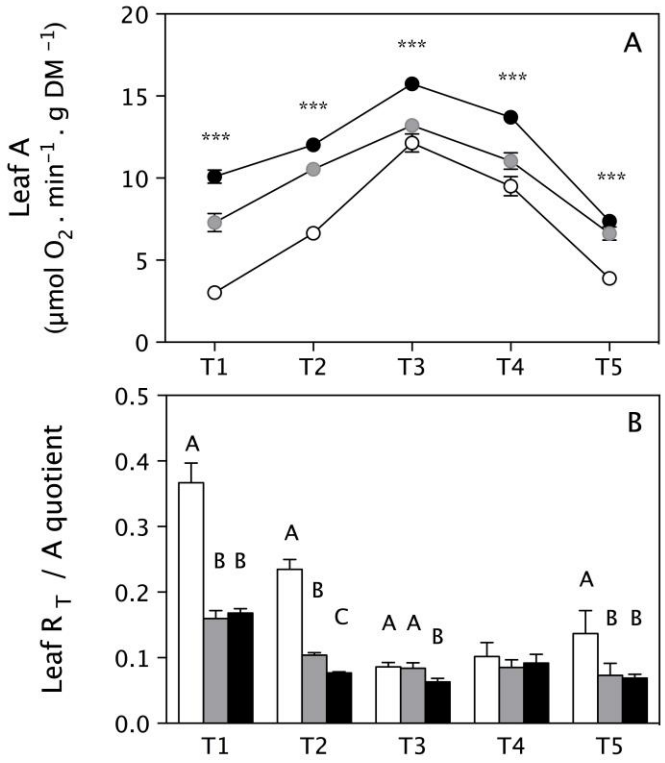


Figure 1-3. Leaf net assimilation (leaf A, µmol O_2. min^{-1}. g DM^{-1}, A) and quotient of leaf total dark respiration to leaf net assimilation (leaf R_T / A quotient, B) of *E. americanum* grown at day/night temperatures of 8/6 ℃ (white), 12/8 ℃ (grey), and 18/14 ℃ (black) during the green leaf period and leaf senescence (T1 to T5). Values for the means ± SEM are presented (n = 5). *** denotes that A differed for at least two out of the three growth temperatures at $P < 0.001$ within each stage. See Table 1-1 for results of multiple test comparisons. Different letters refer to significant differences ($P < 0.05$) among temperatures.

1.3.4 Bulb Respiratory Rates

Bulb respiratory rates are presented on a starch-free dry-mass basis [μmol O_2. min^{-1}. (g starch-free DM)$^{-1}$]. This allowed us to gain a clearer picture of the changes in respiratory rates as a function of metabolite concentrations in the soluble fraction of the cells, given that starch concentrations can reach very high values towards the end of the season (Gandin *et al.*, 2011).

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

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

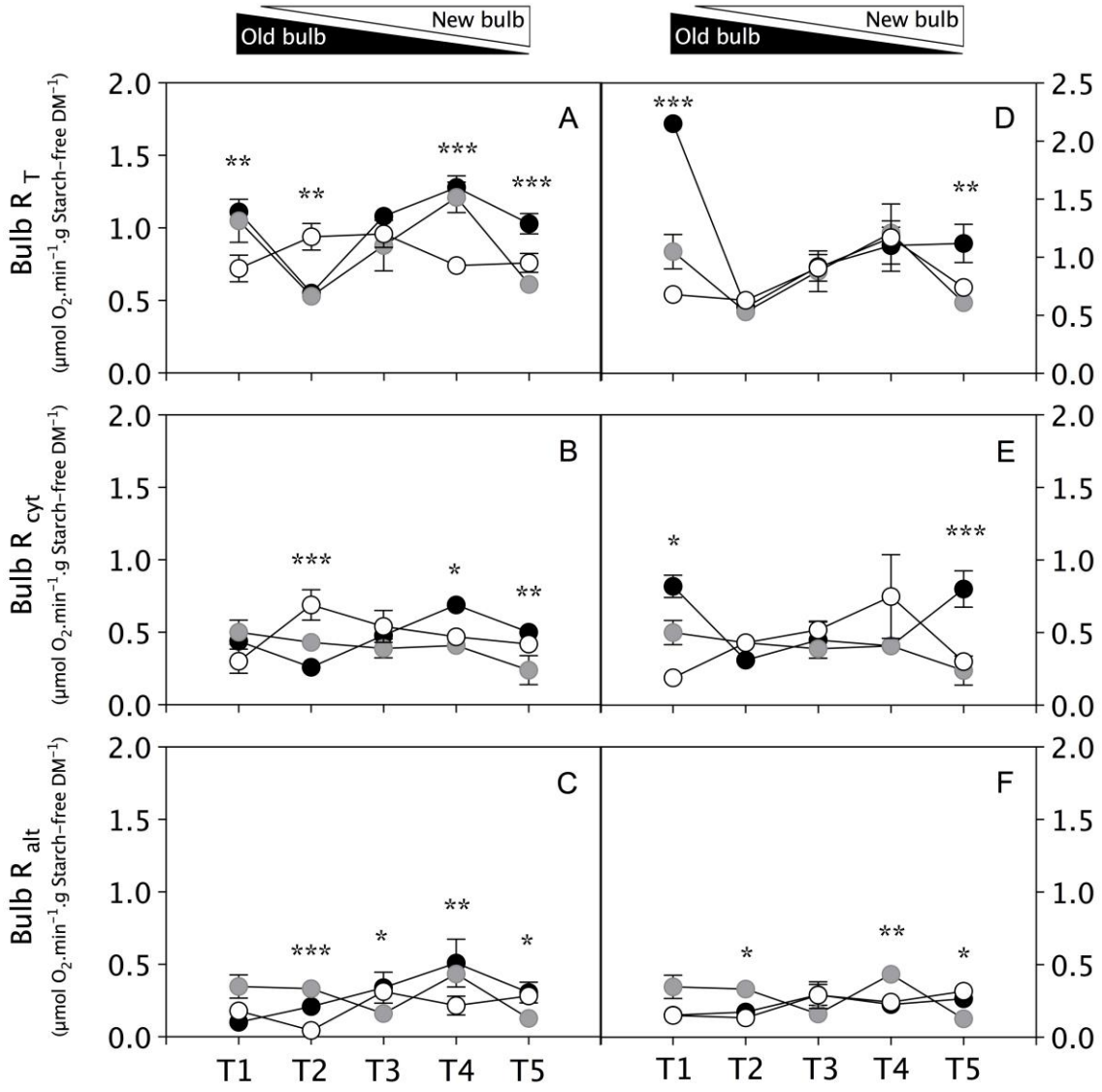


Figure 1-4. Total respiratory rate [bulb R_T, µmol O_2. min^{-1}. (g starch-free DM)$^{-1}$, A and D], capacity of the cytochrome pathway [bulb R_{cyt}, µmol O_2. min^{-1}. (g starch-free DM)$^{-1}$, B and E] and capacity of the alternative pathway [bulb R_{alt}, µmol O_2. min^{-1}. (g starch-free DM)$^{-1}$, C and F] in the bulb of plants grown at day/night temperatures of 8/6 ℃ (white), 12/8 ℃ (grey), and 18/14 ℃ (black) during the green leaf period and leaf senescence (T1 to T5), measured at growth temperature (A – C) and a common temperature 12 ℃ (D – F). Values for the means ± SEM are presented (n = 2 to 4). *, **, and *** denote that the respiratory rates differed for at least two out of the three growth temperatures at $P < 0.05$, < 0.01, and < 0.001, respectively, within each stage. See Table 1-1 for results of multiple test comparisons.

1.3.5 Soluble Sugar Concentrations

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

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

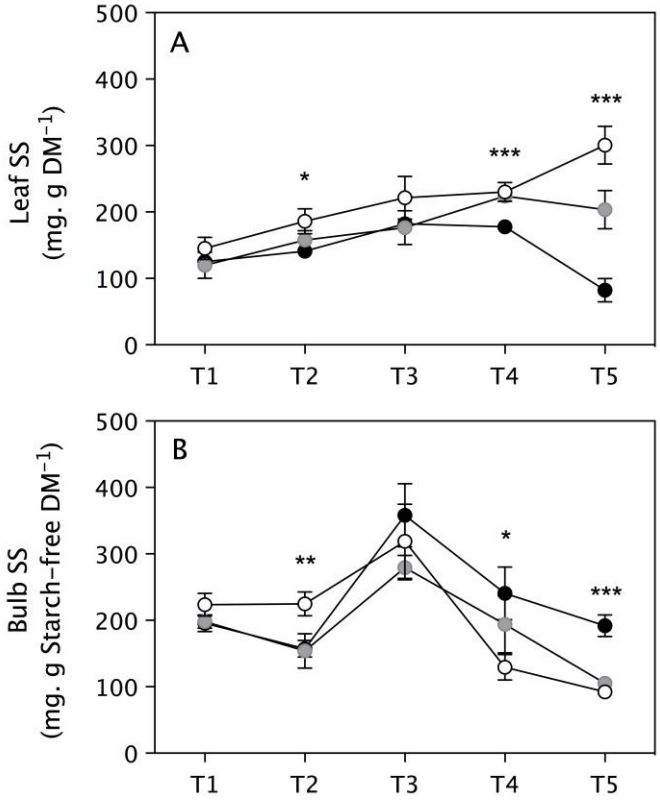


Figure 1-5. Soluble sugar concentrations in the leaf (mg. g DM^{-1}, A) and bulb [mg. (g starch-free DM)$^{-1}$, B] of *E. americanum* plants grown at day/night temperatures of 8/6 ℃ (white), 12/8 ℃ (grey), and 18/14 ℃ (black) during the green leaf period and leaf senescence (T1 to T5). Data are expressed as means ± SEM (n = 5). *, **, and *** denote that the respiratory rates differed for at least two out of the three growth temperatures at $P <$ 0.05, < 0.01, and < 0.001, respectively, within each stage. See Table 1-1 for results of multiple test comparisons.

1.4 Discussion

1.4.1 Thermal Acclimation of Respiration

Leaf R_T of *E. americanum* exhibited homeostasis, which suggests that acclimation occurred in the leaf in response to growth temperature. Similar results of leaf R_T acclimation to changes in growth temperature have been reported in arctic herb *Saxifraga cernua* (McNulty and Cummins, 1987), arctic-alpine *Ranunculus glacialis* (Arnone III and Körner, 1997), and many other species (Loveys *et al.*, 2003). In contrast, in *Vigna radiata* leaves and *Glycine max* cotyledons, plants grown at cool or warm conditions exhibited different R_T when measured at their respective growth temperature, whereas no differences in respiration were observed when measured at the same temperature (González-Meler *et al.*, 1999). Indeed, when respiration was measured at a common temperature of 12 °C, plants grown at the coolest temperature had higher leaf R_T than plants grown at warmer temperatures (Fig. 1-2D), suggesting an improved respiratory capacity to compensate for the slower rates that occur at low temperature. However, it appears that acclimation only occurred in plants grown at 8 °C, as plants grown at either 12/8 °C or 18/14 °C exhibited similar leaf R_T when rates were measured at a common temperature (Fig. 1-2D). This result is consistent with what has been reported in *Saxifraga cernua* (McNulty and Cummins, 1987), where the R_T in cool-grown plants was much higher than in warm-grown plants when measured at an intermediate temperature. Similarly, in the leaves of five temperate ruderal species (Collier and Cummins, 1990) and in *Vigna radiata* hypocotyls (González-Meler *et al.*, 1999), R_T of cooler-grown plants was consistently greater than that from warmer-grown plants at any given measurement temperature.

Thermal acclimation of respiration does not seem to occur in the bulb. Firstly, bulb R_T was sometimes lower, sometimes higher in the plants grown at 8/6 °C than in the two other groups of plants, depending on the phenological stage (Fig. 1-4A), indicating that homeostasis was not reached. Secondly, bulb R_T was very similar among plants grown at the different temperature regimes when measured at a common temperature (Fig. 1-4D). The two instances where R_T differed among growth temperatures (T1 and T5) could not be explained by thermal acclimation, because one would expect thermal acclimation to reduce

R_T in plants grown under higher temperature regimes, whereas we recorded increased R_T values in these plants. There are very few studies reporting respiratory rates of either corm, bulb or tuber as a function of growth temperature; the two we are aware of were done on tissue culture *in vitro*. R_T remains relatively constant in potato callus grown at different temperatures (8 to 28 ℃) when measured at a common temperature (28 ℃), although the capacity of the alternative pathway increases exponentially with growth temperature (Hemrika-Wagner *et al.*, 1983). Yamagishi (1998) also reported similar R_T in bulblets of *Lilium japonicum* Thunb. grown at either 20 or 26 ℃ when measured at a common temperature. Therefore, we conclude that bulb respiration did not acclimate to the different temperature regimes.

Previous work in *E. americanum* has shown that A decreased as growth temperature decreased (Gandin *et al.*, 2011); the same trends were recorded in the current study (Fig. 1-3A). Leaf R_T acclimation at low growth temperature, associated with low A, resulted in high leaf R_T / A quotients at low growth temperature, which suggests that a reduced amount of carbohydrates was available for translocation to the bulb. Despite the reduced amount of C available for translocation, SS remained slightly higher in leaves of plants grown at low temperature than in those of plants grown at the two other temperature regimes, suggesting that not only was there less C available for translocation to the sink, but translocation rates were also most likely slower. Translocation rates have previously been shown to be similar at 12 ℃ and 18 ℃ in another spring geophyte (Badri *et al.*, 2007), but it is possible that lower temperatures, such as 8 ℃, do slow down translocation. For instance, in winter wheat leaves, a proportionally lower C export rate has been found at 5 ℃ than at 20 ℃ (Leonardos *et al.*, 2003). In summary, temperature acclimation of respiration occurred only at the leaf level and only in the plants grown at low temperature. This thermal acclimation of respiration combined with reduced assimilation rates at low temperature, further decreased the amount of C available for translocation to the sink. By reducing the amount of C available for translocation, plants grown at low temperature would establish a better equilibrium between source and sink activity, prolonging leaf life duration until feedback inhibition of photosynthesis would induce leaf senescence.

We initially hypothesized that R_{alt} would be involved in the modulation of R_T at both the leaf and bulb level as a response to temperature; yet similar low values for leaf R_{alt} were observed in plants grown at the different temperature regimes, whereas the growth temperature effect on bulb R_{alt} was not consistent. Similar values for leaf R_{alt} among growth temperatures suggested that leaf R_{alt} thermally acclimated, but differences were not large enough to be detected when measured under a common temperature. Similar results were observed in white spruce (*Picea glauca*) roots, where R_{alt} was not affected by growth temperatures or measurement temperatures between 4 and 18 ℃, and represented 23 % of the total capacity of electron transport (Weger and Guy, 1991). However, the stimulation of R_{alt} at low growth temperatures has been reported in leaves of the perennial herb *Saxifraga cernua* (McNulty and Cummins, 1987) and of several temperate ruderal species (Collier and Cummins, 1990). The fact that growth rate was much less affected by low temperature in *E. americanum* (Gandin *et al.*, 2011) than in the species cited above might partly explain why leaf R_{alt} was not strongly stimulated at low temperatures. When sink activity is decreased, C accumulates in the leaves, where it could stimulate R_{alt}. Gandin *et al.* (2009) have shown that bulb R_{alt} can be strongly stimulated when *E. americanum* plants were grown under elevated CO_2 concentrations (high source activity), indicating that bulb R_{alt} can be modulated in response to source–sink imbalance. In warmer-grown plants, where C translocation was most likely higher than in cool-grown plants, we did not detect a consistent increase in bulb R_{alt}. We conclude that phenological stage has a stronger impact on the bulb respiratory rates than growth temperature.

1.4.2 Respiration as A Function of Phenological Stage

Numerous studies have shown that plant respiration is modulated during organ development, with higher rates of respiration in young compared with mature tissues (Azcon-Bieto *et al.*, 1983; McDonnell and Farrar, 1993; Atkin and Cummins, 1994; Armstrong *et al.*, 2006). The rate of respiration is often linearly related to relative growth rates of the tissues, which reflects modulation in the production of energy and carbon skeletons to fulfil the needs for biosynthesis and cellular maintenance (Lambers *et al.*, 1998). In *E. americanum*, leaf R_T was relatively constant throughout the period leading up to senescence (Table 1-1), which would indicate that most of leaf growth was already

achieved at T1. Nevertheless, leaf R_{cyt} was higher at T1, then decreased to reach a stable level up to the beginning of leaf senescence. Florez-Sarasa *et al.* (2007) previously demonstrated that growth respiration of *Arabidopsis thaliana* rosette leaves was largely dependent on the activity of the cytochrome pathway, which would suggest that growth processes requiring ATP still occurred at T1 in the leaf of *E. americanum*. We expected bulb R_{cyt} to be high at T2 while cells were actively dividing within the new bulb, but this was not the case, except at the coolest growth temperature (Table 1-1). At T2, the new bulb was very small; we posit that most respiration originated from the old bulb that was being emptied by then and was less metabolically active.

Unlike leaf R_T, bulb R_T exhibited a general increase through time, as the new bulb increased in size and accumulated starch, except at the coolest growth temperature. The current results bring support to the hypothesis that bulb R_T increases as sink limitation builds up through time. Under high CO_2, where sink limitation was stronger, the increase in R_T over time was more pronounced than under ambient CO_2 (Gandin *et al.*, 2009), also pointing toward a modulation of bulb R_T by source–sink imbalance. Steingröver (1981) reported that R_{alt} was higher during early growth than during extensive SS and biomass accumulation in the taproot of carrot (*Daucus carota* L.). Similarly, R_{alt} decreased during inulin accumulation in storage roots of *Hypochaeris radicata* (Lambers and Van der Dijk, 1979). These authors concluded that « This suggests that sugars are oxidized 'wastefully' only if sugar supply from the shoots is in excess of the amount than can be utilized for energy production, for structural growth or for storage ». In plants where most of the growth of the perennial organ takes place later in the season, long after the perennial organ has been developed, sink limitation is more likely to occur early in the season (Steingröver, 1981). On the other hand, in spring ephemerals where growth and storage occur concomitantly, sink limitation is most likely to build up through time as growth slows down. However, regardless of the timing of sink limitation, R_T of the perennial organ appears to be modulated by source–sink balance. The improved balance between source and sink activity in cool-grown plants of *E. americanum* most likely explains why bulb R_T did not steadily increase with time in these plants compared with those grown at higher temperatures.

As the respiratory rates that we measured were completely dependent upon endogenous substrate levels, bulb R_T could also vary with carbohydrate availability (Atkin and Tjoelker, 2003), which originated either from C stored in the old bulb or C translocated from the leaf. Changes in bulb R_T and bulb SS concentrations were not always related to each other, given that bulb SS concentrations started decreasing between T3 and T4, while bulb R_T continued to increase up to T4 (Figs. 1-4A and 1-5B). At the lowest growth temperature, modulation of bulb SS concentrations and bulb R_T over time did not match one another. Similarly, the reduction in R_T in taproot during carbohydrate storage was not accompanied by a reduction in reducing sugar concentrations in *Hypochaeris radicata* (Lambers and Van der Dijk, 1979), nor in carrot (Steingröver, 1981). Other researchers have also reported conflicting results between carbohydrate levels within a tissue and either R_T (Atkin *et al.*, 2000b) or R_{alt} (Wang *et al.*, 2011). Taking into account the relative growth rate of the storage organ, i.e., its sink strength, appears to better explain the modulation of its respiratory rates over time than soluble sugar availability.

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

1.5 Acknowledgements

This research was financially supported by a Discovery grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to LL. The authors gratefully acknowledge Mr. François Larochelle for his expert technical assistance in growth chamber experiments, Mr. Gaétan Daigle for advice on statistical analyses, and Dr. William F.J. Parsons for English language revision. The authors also sincerely thank all of the colleagues in the laboratory who participated in plant harvesting: Moustafa A. Khalf, Julie Bussières, Pierre-Paul Dion, Jade Boulanger-Pelletier, Karolane Pitre and Dominique Manny.

Table 1-1. Summary of two-way ANOVA on the effects of stage (T1 to T5) and growth temperature (8/6 ℃, 12/8 ℃ and 18/14 ℃) on leaf and bulb respiratory rates (R_T, R_{cyt} and R_{alt}), leaf net assimilation (A), leaf dark respiration to assimilation ratio (leaf R_T / A), and leaf and bulb soluble sugar (SS) concentrations.

Variables	GT (℃)	MT (℃)	*F*-values			Multiple comparisons					
			T℃	Stage	T℃ × Stage	for Stage only [1] and T℃ × Stage					for T℃ only [2]
						T1	T2	T3	T4	T5	
Leaf R_T	8/6	8									
	12/8	12	0.5	12.0***	0.8	a	a	a	a	b	
	18/14	18									
	8/6	12									A
	12/8	12	6.0**	12.2***	0.2	a	a	a	a	b	B
	18/14	12									B
Leaf R_{cyt}	8/6	8									B
	12/8	12	4.3 *	31.7***	1.5	a	b	b	b	c	B
	18/14	18									A
	8/6	12									
	12/8	12	0.4	18.7***	1.0	b	a	bc	cd	d	
	18/14	12									
Leaf R_{alt}	8/6	8									
	12/8	12	0.9	6.3***	1.6	ab	ab	a	a	b	
	18/14	18									
	8/6	12				c B	a A	b	ab	c	
	12/8	12	0.0	20.5***	2.9 *	a AB	a B	a	a	b	
	18/14	12				a A	a C	a	a	b	
Leaf A	8/6	8				e C	c C	a B	b B	d B	
	12/8	12	230.1***	187.3***	16.4***	c B	b B	a B	b B	c A	
	18/14	18				d A	c A	a A	b A	e A	

Table 1-1. (continued)

Variables	GT (℃)	MT (℃)	*F*-values			Multiple comparisons					
			T℃	Stage	T℃ × Stage	for Stage only [1] and T℃ × Stage					for T℃ only [2]
						T1	T2	T3	T4	T5	
Leaf R_T / A	8/6	8				a A	b A	d A	cd	c A	
	12/8	12	52.5***	57.2***	6.9***	a B	b B	b A	b	c B	
	18/14	18				a B	bc C	d B	b	cd B	
Leaf SS	8/6	-				c	bc A	b	b A	a A	
	12/8	-	21.8***	19.9***	4.2 **	c	bc AB	ab	a A	ab B	
	18/14	-				b	b B	a	a B	c C	
Bulb R_T	8/6	8				B	A		B	B	
	12/8	12	9.1**	9.26 **	7.4***	ab AB	d B	bc	a A	cd B	
	18/14	18				ab A	c B	b	a A	b A	
	8/6	12				B				B	
	12/8	12	6.6 **	9.0 **	3.2 *	ab B	d	bc	a	cd B	
	18/14	12				a A	c	b	b	b A	
Bulb R_{cvt}	8/6	8				b	a A	a	a B	ab A	
	12/8	12	1.0	1.8	4.8 **		AB		B	B	
	18/14	18				b	c B	b	a A	b A	
	8/6	12				b B	ab	a	a	ab B	
	12/8	12	2.5	0.8	3.7 **	AB				B	
	18/14	12				a A	b	b	b	a A	
Bulb R_{alt}	8/6	8				a	b B	a A	a B	a A	
	12/8	12	2.6	3.2 *	7.0***	a	a A	b B	a A	b B	
	18/14	18				b	ab AB	ab A	a A	ab A	
	8/6	12				b	b B	ab	ab B	a A	
	12/8	12	0.2	1.5	6.4***	a	a A	b	a A	b B	
	18/14	12					AB		B	AB	

Table 1-1. (continued)

Variables	GT (℃)	MT (℃)	*F*-values			Multiple comparisons					
			T℃	Stage	T℃ × Stage	for Stage only [1] and T℃ × Stage					for T℃ only [2]
						T1	T2	T3	T4	T5	
Bulb SS	8/6	-				a	a ^{A}	a	b ^{B}	b ^{B}	
	12/8	-	4.8 *	30.5***	6.6***	ab	bc ^{B}	a	ab ^{AB}	c ^{B}	
	18/14	-				b	b ^{B}	a	ab ^{A}	b ^{A}	

Note: Bulb respiratory rates and bulb SS concentrations are presented on a starch-free bulb dry-mass basis. *F*-values are presented, followed by significance level (*, **, and *** denote a significant difference at $P < 0.05$, < 0.01, and < 0.001, respectively). Multiple comparisons among temperatures and among stages are also shown. Lowercase letters denote significant differences ($P < 0.05$) among stages (comparisons in each row), and uppercase letters refer to significant differences ($P < 0.05$) among temperatures (comparisons in each column). Absence of lowercase letters in rows (stage comparisons) and uppercase letters in columns (temperature comparisons) indicates non-significant differences. GT, growth temperature; MT, measurement temperature; T℃, temperature.

[1] Multiple comparisons for "stage effect" were shown only when stage effect was significant, but the interaction effect from T℃ × stage was not statistically significant.

[2] Multiple comparisons for the effect from T℃ were shown only when T℃ effect was significant, but the interaction effect from T℃ × stage was not statistically significant.

Chapitre 2. Effect of growth temperatures on carbon partitioning (non-structural *vs.* structural) in the bulb of a spring geophyte *Erythronium americanum*

Yanwen Dong[1,2], Dominique Gérant[2], Isabelle Ziegler-Devin[3] and Line Lapointe[1]

[1] Département de Biologie and Centre d'Étude de la Forêt, Université Laval, Québec, QC, Canada

[2] Unité Mixte de Recherches 1434 SILVA, Université de Lorraine/INRA/AgroParisTech, Faculté des Sciences et Technologies, 54506 Vandœuvre-lès-Nancy, France

[3]LERMaB, EA 4370, Université de Lorraine, Faculté des Sciences et Technologies, F-54506 Vandœuvre-lès-Nancy, France

Résumé

L'allocation du carbone (C) des sources aux puits et la répartition du C vers les différentes réserves de C sont régulées par les priorités des exigences des plantes, mais aussi par les conditions de croissance qui peuvent à leur tour influencer la croissance et la performance des plantes. De nombreuses études ont montré une biomasse accrue des organes puits chez les éphémères printanières cultivées à plus basse température. Une analyse précédente sur le corme du crocus a également révélé un investissement de C plus faible dans le matériau de la paroi cellulaire dans des conditions plus fraîches à la fin de saison. Nous avons émis l'hypothèse que, pour l'*Erythronium americanum*, l'activité prolongée de puits chez les plantes cultivées à basse température pourrait être liée à des modifications de la répartition du C entre les composés non-structuraux et les composants de la paroi cellulaire. Des pools de C non-structuraux et structuraux ont été analysés au niveau du bulbe des *E. americanum* cultivés à trois régimes de température (8/6 °C, 12/8 °C et 18/14 °C, jour/nuit). La position dominante de l'amidon en tant que principale réserve dans le bulbe a été attestée, et les fructanes ou glucomannanes étaient pratiquement absents. À haute température, un investissement plus important de C dans les composants de la paroi cellulaire, reflétant à la fois dans la fraction d'hémicelluloses et de 'cellulose et lignine', confirme l'hypothèse d'un ajustement de la répartition du C entre les pools de C structuraux et non-structuraux dans le bulbe une fois les cellules étaient pleines d'amidon afin de maintenir l'activité du puits. Des influences négatives sur la croissance des plantes et le bilan de C pourraient se produire lorsqu'il y avait davantage de C investi dans la fraction de 'cellulose et lignine', alors qu'une plus abondante répartition de C vers la fraction d'hémicelluloses semblait être un meilleur moyen de maintenir l'équilibre source−puits à cause de la fonction potentielle de réserve des hémicelluloses.

Mots-clés: répartition du carbone, glucides non-structuraux, glucides structuraux, *Erythronium americanum*, paroi cellulaire, hémicelluloses, cellulose et lignine, pectines

Abstract

Carbon (C) allocation from sources to sinks and C partitioning into the different C pools are regulated by priorities of requirements in plants, but also by growth conditions which may in turn influence plant growth and performance. Numerous studies have shown enhanced sink organ biomass under cooler conditions in spring ephemerals. Previous analysis in crocus corm also found a reduced C partitioning into the cell wall material under cooler condition at the end of the season. We hypothesized that, for *Erythronium americanum*, the extended sink activity in cool-grown plants might be related to modifications of C partitioning between non-structural carbohydrates and cell wall components. Both non-structural and structural C pools within the bulb of *E. americanum* grown at three temperature regimes (8/6 ℃, 12/8 ℃, and 18/14 ℃, day/night) were analyzed. The dominant position of starch as the primary reserve within the bulb was attested, and fructans or glucomannans were basically absent. C partitioning into the different carbohydrates fractions were modified by growth temperature. At warmer temperature, a greater C investment in cell wall components, reflected in both hemicelluloses and 'cellulose and lignin' fractions, support the hypothesis of an adjustment of C partitioning between non-structural and structural C pools in the bulb once the cells were full of starch in order to maintain sink activity. Negative influences on plant growth and C budget would occur when more C is invested in 'cellulose and lignin' fraction, whereas greater C partitioning into hemicellulose fraction seemed a better way to maintain source–sink balance due to the potential reserve function of hemicelluloses.

Keywords: Carbon partitioning, non-structural carbohydrates, structural carbohydrates, *Erythronium americanum*, cell wall, hemicelluloses, cellulose and lignin, pectins

2.1 Introduction

Spring ephemerals are perennial plants present in the understory of deciduous forests. They are characterized by a very short growth period of both leaf and new perennial organ, bulb or corm according to the species (Lapointe, 2001). However, we have shown that leaf life duration can be modulated by growth temperature, along with final biomass of the perennial organ. In the spring ephemerals *E. americanum* (Lapointe and Lerat, 2006; Gandin *et al.*, 2011) and *Crocus vernus* (Badri *et al.*, 2007; Lundmark *et al.*, 2009), the final biomass of the perennial organ is higher at cooler compared to higher growth temperatures. It was suggested that, at cool temperature, the larger size of the perennial organ was not explained by modifications of the activity of the source at cool temperature: comparable $^{14}CO_2$ incorporation after 1h pulse-chase (Lundmark *et al.*, 2009) and even lower net assimilation (on a leaf area basis) (Gandin *et al.*, 2011), but rather by a prolonged leaf longevity (Badri *et al.*, 2007; Lundmark *et al.*, 2009; Gandin *et al.*, 2011). Although sink biomass is supposed to be a consequence of leaf activity, Lapointe (2001) proposed the alternative hypothesis that, in spring ephemeral, a threshold sink demand is required to maintain leaves active, otherwise irreversible leaf senescence is induced. According to this hypothesis, the sink activity was extended at cool temperature, which allowed a longer epigeous growth period. Characterizing sink activity requires to study not only total biomass accumulation but also C partitioning among the main C pools, which are non-structural (NSC: starch, sugars, and other derived compounds such as organic acids and amino acids) and structural carbohydrates (SC: cell wall polysaccharides), whose concentrations are known to vary according to environmental conditions (Poorter and Villar, 1997).

Starch is the major NSC stored in the perennial organ(s) (bulb or corm) of many geophytes. Final starch concentrations in the bulb [on a dry mass (DM) basis] can reach 65 % for *Lilium longiflorum* (Miller and Langhans, 1990), and nearly 88 % for *Erythronium americanum* (Gandin *et al.*, 2011). In geophytes, other carbohydrates may also be stored in the sink organs during the leafy period, though the large fraction of starch may hide their role as C reserves. Indeed, fructans, fructose-based polymers with a terminal glucose unit, are commonly found in the perennial organs of the Liliaceae, Alliaceae,

Amaryllidaceae, Hyacinthaceae, Iridaceae, and other geophytic families (Miller, 1992; Hendry and Wallace, 1993; Ranwala and Miller, 2008). Fructans have been recognized as protective agents against abiotic stresses and have been involved in chilling and drought tolerance (Valluru and Van den Ende, 2008; Livingston *et al.*, 2009).

In *Crocus vernus*, Lundmark *et al.* (2009) reported an increased partitioning of C into the cell walls of the corm in the late season, which suggested a change in the pattern of C partitioning into SC pools once the cells were filled with starch. Plant cells grow by expansion of their cell walls through the synthesis of different polysaccharides and their integration into an organised network (Cosgrove, 2005). In monocot plants, the primary wall (on a DM basis) is composed of cellulose (20−30 %), hemicelluloses (10−25 %), pectins (5 %), structural proteins (1 %), and phenolic compounds (1−5 %) (Pauly and Keegstra, 2008; Vogel, 2008; Schädel *et al.*, 2010a). In the secondary walls, in addition to cellulose and hemicelluloses, lignin deposition can account for 7 to 20 % (Pauly and Keegstra, 2008; Vogel, 2008), which serves to stiffen them. Cellulose microfibrils are chemically stable and insoluble (Ochoa-Villarreal *et al.*, 2012). Hemicelluloses include four main classes: xyloglucans, xylans, mannans, and glucomannans (Ebringerova *et al.*, 2005; Scheller and Ulvskov, 2010), and they are associated with different taxonomic groups (Buckeridge *et al.*, 2000; Li *et al.*, 2005; Gille *et al.*, 2011; Pellny *et al.*, 2012; Pauly *et al.*, 2013). In geophytes, an accumulation of glucomannans, either in their seeds or in their perennial organs, probably serves as an alternative reserve polysaccharide in addition to starch (Andrews *et al.*, 1956; Matsuo and Mizuno, 1974; Meier and Reid, 1982; Miller, 1992; Ranwala and Miller, 2008). Thus, hemicelluloses are structural components of the cell wall, but unlike cellulose, they have also been considered as storage compounds that can be mobilized for growth processes (Crawshaw and Reid, 1984; Reid, 1985; Buckeridge *et al.*, 2000). Pectins, the third group of primary cell wall polysaccharides, contain high levels of different galacturonans. Pectins are not considered as storage compounds, but are known to be involved in specific developmental modifications, such as cell wall swelling and softening during fruit ripening (Fischer and Bennett, 1991), and root cap cell differentiation (Wen *et al.*, 1999).

Modifications of the cell wall composition are an important element of adaptive responses to abiotic stress (Le Gall *et al.*, 2015). Thus, in *E. americanum*, the larger bulb biomass at cooler compared to higher growth temperatures could potentially be explained by partitioning C into additional non-structural carbohydrates (e.g. fructans), and also into cell walls components, which would induce extended sink ability to mobilize photo-assimilates. To test this hypothesis, we analyzed the qualitative and quantitative modifications of both NSC and SC pools within the final bulb biomass of *E. americanum* grown at three growth temperatures (8/6 ℃, 12/8 ℃, and 18/14 ℃, day/night). We developed a method that combined the protocols from Ranwala and Miller (2008) to analyze the composition and concentrations of NSC and SC compounds by High-Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD), and from Schädel *et al.* (2010a) to analyze the composition of the structural pools. C contents of the initial organic matter and of each monosaccharide or polysaccharide purified were used to determine the effect of the three growth temperatures on C partitioning within the bulbs of *E. americanum*.

2.2 Material and Methods

2.2.1 Plant Material and Experimental Design

In September 2013, bulbs of *E. americanum* of similar diameter (from 6 to 8 mm diameter) were collected from a maple forest near Saint-Augustin-de-Desmaures (QC, Canada; 46°48'N, 71°23'W). About 500 bulbs planted individually in 10-cm-diameter plastic pots, containing Turface (calcined clay granules, Applied Industrial Materials Corp., Buffalo Grove, IL, USA) as the substrate. After about 20 days at 10 ℃ in the dark, which allowed root growth, the pots were placed in a cold room (4−5 ℃) for five months for cold stratification. Substrate moisture content was checked weekly, and the pots were watered when the top 5 cm of Turface had dried. At the beginning of March 2014, all the pots were moved to a growth chamber that was set at 8/6 ℃ (day/night) in darkness for about one week of acclimation. Once the shoots became visible, the pots were then randomly distributed into three growth chambers (PGW36, Conviron Inc., Winnipeg, MB, Canada) under the following light conditions: photoperiod of 14 h and a photosynthetic photon flux

density (PPFD) of 300 µmol.m^{-2}.s^{-1}. The temperature regimes that were used in the experiment were based upon our previous study (Dong *et al.*, 2018): three temperature regimes (day/night) of 8/6 °C, 12/8 °C, and 18/14 °C were tested, with relative humidity of 50 %, 65 %, and 75 %, respectively. Relative humidity was modulated as a function of temperature to maintain a constant vapour pressure deficit (VPD) among the growth chambers. Plants were watered regularly, and fertilized weekly with 10 % Hoagland's solution for optimal growth (Lapointe and Lerat, 2006).

2.2.2 Bulb Harvest

Bulbs were harvested at the end of the season after the unique leaf has senesced and they had entered dormancy. Five plants per temperature regime were collected and stored at -80 °C until freeze-drying for 48 h. Dry material was weighed separately for new bulbs and mother bulbs, and they were ground together into fine powder in a ball mill (Qiagen Inc. Toronto, Ontario, Canada), since the dry material of mother bulbs (remaining debris) were too low for a separate analysis.

2.2.3 Soluble Sugars and Starch Determination

Soluble sugars and starch were extracted from 10 mg of bulb dry matter with 650 µL of methanol/chloroform/water (12:5:3, v/v), then the extracts were centrifuged at 17 000 g for 10 min at 4 °C. This step was repeated twice and the resulting supernatants were pooled together. Total soluble sugar concentration was determined on this soluble fraction by colorimetry as described by Hansen and Møller (1975). An aliquot of 100 µL of the soluble fraction was mixed with 1.5 mL of anthrone, incubated at 60 °C for 20 min then cooled (on ice then at room temperature). Soluble sugar concentration was then determined colorimetrically at 620 nm, with glucose used as standard. The final pellet after methanol/chloroform/water extraction was dried for 1 h at 30 °C. After addition of 500 µL HCl 2 % (v/v), incubation at 100 °C for 2 h then cooling, the extracts were centrifuged at 17000 g for 5 min. The concentration of glucose released by starch hydrolysis and recovered in the supernatant was also determined colorimetrically by anthrone, using 100 µL of the soluble fraction, similarly as soluble sugars.

2.2.4 Purification of the Structural and Non-Structural Compounds

The sequential protocol was adapted from Ranwala and Miller (2008) and Schädel *et al.* (2010a). The different steps are summarized in the extraction scheme (Fig. 2-1).

Step 1. Fifty mg of bulb powder were extracted at 70 °C with 80 % ethanol (three extractions of 3 mL each, 30 min per extraction). Tissue suspensions were centrifuged at 4000 g for 10 min after each extraction, and the supernatants were combined. The extracts were evaporated at 55 °C under vacuum, and dissolved in 11.5 mL of HPLC-grade water. These fractions were used to determine the composition and concentrations of soluble carbohydrates (mono- and disaccharides and short-chain fructans) by High-Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) (see below).

Step 2. The ethanol-insoluble fractions were extracted at 70 °C with HPLC-grade water (two extractions of 3 mL each, 30 min per extraction) and were centrifuged at 10 000 g for 10 min after each extraction, and the supernatants were combined. The soluble extracts were made up to 11.5 mL with HPLC-grade water. Two kinds of acid hydrolysis of the extracts were performed to separate long-chain fructans and glucomannans. According to the results on the optimum acid concentration and duration for hydrolysis tested by Ranwala and Miller (2008), the soluble extracts were boiled for 5 min with 0.05 M HCl for long-chain fructans determination, and for 30 min with 2 M HCl for glucomannans determination. These fractions were used to determine by HPAE-PAD the composition and concentrations of long-chain fructans and glucomannans.

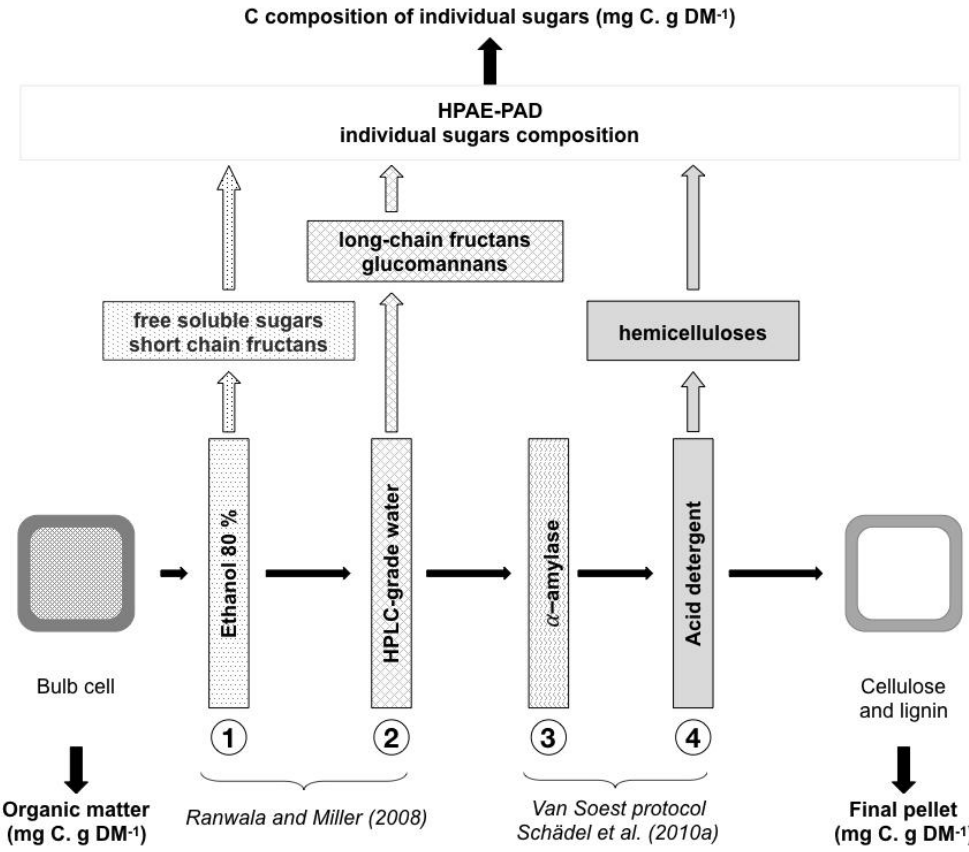


Figure 2-1. Schematic depiction of sequential protocols adapted from Ranwala and Miller (2008) and Schädel *et al.* (2010a) for the purification of the structural and non-structural compounds.

Step 3. The remaining residue that was insoluble in ethanol or HPLC-grade water was used to determine hemicelluloses concentrations according to the modified micro-extraction method described by Schädel *et al.* (2010a), based on Van Soest fiber analysis (Van Soest, 1963; Van Soest and Wine, 1967). After addition of 1.5 mL of α-amylase solution (EC:3.2.1.1; 15 U.mg solid^{-1}), the insoluble residue was incubated at 85 °C for 30 min, then centrifuged at 13 000 g for 10 min. The remaining pellet was then washed with distilled water, and this starch-free residue was dissolved in 1.5 mL of a neutral detergent containing 18 mM sodium tetraborate decahydrate, 66 mM EDTA (Ethylenediaminetetraacetic acid), 10.4 mM SDS (Sodium dodecyl sulfate), 10 mL.L^{-1} triethylene glycol and 32 mM dibasic sodium phosphate to extract the remaining soluble components and soluble pectins. Sodium sulfite (0.5 mg.g^{-1} solid) was then immediately added to each sample to eliminate proteins.

Step 4. After centrifugation, the pellet containing hemicelluloses, cellulose and lignin was washed twice with hot deionized water, once with acetone and finally once with deionized water. The washed residue was then dried overnight in Speed-Vac at 60 °C, and weighed on a precise balance (on a 0.01 mg scale). A solution of an acid detergent containing 1N H_2SO_4 and 20 g.L^{-1} of hexadecyl trimethylammonium bromide, and 55 mM of demineralized water was added to the dry pellet. The samples were then placed in a dry bath at 100 °C for 1 h to hydrolyze the hemicelluloses. Each extract was then centrifuged at 13000 g for 10 min to collect a supernatant containing the monosaccharides from hemicellulose hydrolysis, and a pellet which contained 'cellulose and lignin' fraction. The supernatants were evaporated at 55 °C under vacuum, and dissolved in HPLC-grade water. Monosaccharides concentrations and compositions were determined with HPAE-PAD. The final pellets ('cellulose and lignin' fraction) were dried in Speed-Vac at 60 °C overnight, then weighed. Total hemicellulose concentrations were gravimetrically calculated as the difference between the total structural fraction (the pellet obtained after Step 3) and 'cellulose and lignin' fraction (final pellet).

2.2.5 HPAE-PAD Analyses

Monosaccharides and soluble polysaccharides concentrations of the different fractions were analyzed by high-performance anion-exchange chromatography (HPAE) coupled with pulsed amperometric detection (PAD) (ICS-3000 Dionex), equipped with a Dionex CarboPac^{TM} PA-20 (3×150 mm) analytical column. Filtered samples (20 µL) were eluted at 35 °C and at 0.4 mL.min^{-1} with the following composition: pure water 99.2 % / 250 mM NaOH 0.8 %: 0 to 20 min; pure water 75 % / 250 mM NaOH 20 % / NaOAc (1M)-NaOH (20 mM) 5 %: 20 to 37 min; pure water 40 % / 250 mM NaOH 20% / NaOAc (1M)-NaOH (20 mM) 40 %: 37 to 41 min. Each elution was followed by a wash and subsequent equilibration time. External sugar and uronic acid standards were used for calibration (7 points per curve): fucose, glucose, xylose, galactose, mannose, rhamnose, arabinose, and galacturonic acid, glucuronic acid, fructose and sucrose (all provided by Sigma-Aldrich).

2.2.6 Carbon Analyses and Calculations

Concentrations of all the purified fractions (mg. g DM^{-1}) were calculated according to the biomass of the bulb used for the extraction and to the different volumes and dilutions during the purification processes. Due to the high concentration of starch within the bulb, concentrations of free soluble sugars and of structural carbohydrates were also expressed on a starch-free DM basis [mg. g (starch-free DM)$^{-1}$]. C and nitrogen (N) concentrations were measured after combustion of aliquots of freeze-dried initial powders (total C), and of final pellets (‘cellulose and lignin’ fraction) with an elemental analyzer (NCS 2500, ThermoQuest, Milan, Italy). C concentrations of the purified monosaccharides and soluble polysaccharides identified by HPAE-PAD were calculated according to the carbon percentage contained in each molecule, for example, glucose ($C_6H_{12}O_6$): 39.96 %, sucrose ($C_{12}H_{22}O_{11}$): 42.07 %, arabinose from hemicelluloses ($C_5H_{10}O_5$): 39.97 %, etc.. The biomass of the bulbs was then used to calculate the amounts of C (expressed in mg C) in the different fractions. Finally, C partitioning corresponds to the amount of C in one fraction expressed in percent of the total starch-free C amount of the bulb. N concentrations were also measured after combustion of aliquots of freeze-dried initial powders (total N),

and of final pellets (‘cellulose and lignin’ fraction) with the elemental analyzer to validate our purification (cf. discussion).

2.2.7 Statistical Analysis

One-way ANOVAs were carried out to test the effect of growth temperature on concentrations of NSC (starch and free soluble sugars) and SC (‘cellulose and lignin’ fraction and hemicelluloses) present in the bulb, ratio of ‘cellulose and lignin’ fraction to hemicellulose fraction, composition of each monosaccharide of hemicelluloses, and C partitioning to each carbohydrate fraction. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), and graphs were generated with Prism 7.0 for Mac (Graphpad Software Inc., La Jolla, CA, USA).

2.3 Results

2.3.1 Bulb Biomass

The final DM of the new bulb was much higher than that of the mother bulb, about 28-fold, 25-fold, and 23-fold for plants grown at 8/6 ℃, 12/8 ℃, and 18/14 ℃, respectively (Table 2-1). Growth temperature significantly affected the final DM of the new bulb, which reached much higher values in cooler-grown plants than in warmer-grown plants (P = 0.002). The final mother bulb DM did not exhibit significant differences among growth temperatures (P = 0.059).

Table 2-1. Bulb biomass (mg), and concentrations (mg.g DM^{-1}) of non-structural carbohydrates (starch and free soluble sugars) and structural carbohydrates ('cellulose and lignin' fraction and hemicelluloses) present at the end of the growth period in the bulb of *E. americanum* grown at day/night temperatures of 8/6 ℃, 12/8 ℃, and 18/14 ℃. The ratio of the 'cellulose and lignin' fraction to hemicelluloses are presented for each temperature regime. Concentrations of structural carbohydrates are also expressed on a starch-free basis [mg.g (starch-free DM) $^{-1}$]. Values are means ± standard error of the mean (SEM) (n = 5). Results of one-way ANOVAs (F values and P values) testing the effect of growth temperature are presented. Different letters in rows refer to significant differences (P < 0.05) among growth temperatures. Absence of letters in rows indicates non-significant differences.

	8 / 6 ℃		**12 / 8 ℃**		**18 / 14 ℃**		F value	*P* value
Bulb biomass (mg)								
New bulb	244.0 ± 17.0	a	188.5 ± 22.3	a	138.1 ± 18.8	b	11.53	**0.002**
Mother bulb	8.7 ± 0.6		12.0 ± 0.8		10.0 ± 1.3		3.62	*0.059*
Non-structural carbohydrates (mg . g DM $^{-1}$)								
Starch	685 ± 22.2	b	796.5 ± 39.5	a	852.9 ± 39.7	a	13.99	**< 0.001**
Free soluble sugars	18.1 ± 0.7		16.5 ± 0.9		17.0 ± 1.0		0.94	*0.416*
Structural carbohydrates (mg . g DM $^{-1}$)								
Cellulose and lignin	49.7 ± 5.5	a	32.7 ± 5.9	b	35.5 ± 4.2	b	7.40	**0.008**
Hemicelluloses	51.4 ± 3.8	a	36.4 ± 3.9	b	39.2 ± 4.4	b	8.95	**0.004**
'Cellulose and lignin': Hemicelluloses ratio	1.0 ± 0.1		0.9 ± 0.1		0.9 ± 0.1		0.21	*0.817*
Structural carbohydrates [mg . g (starch-free DM) $^{-1}$]								
Cellulose and lignin	162.7 ± 24.2		176.7 ± 27.6		247.2 ± 26.0		3.61	*0.059*
Hemicelluloses	165.1 ± 13.4	b	194.3 ± 24.1	ab	275.7 ± 25.0	a	5.47	**0.021**

2.3.2 Non-Structural and Structural Carbohydrate Concentrations

As the main form of NSC in the bulb, starch concentrations significantly differed among growth temperatures ($P < 0.001$, Table 2-1), where the lower values were observed at the coolest growth temperatures. Free soluble sugars (SS) fraction contained only sucrose (data not shown) which concentrations did not differ among all three growth temperatures ($P = 0.416$). The concentrations of free soluble sugars were nearly 40-fold lower than starch concentrations in plants grown at 8/6 ℃, and about 50-fold lower at two warmer temperatures. The analysis of individual sugar composition for steps 1 and 2 (Fig. 2-1) did not detect the presence of mannose, which suggested that glucomannans are most likely absent in the bulb of *E. americanum*. Total fructan concentrations were determined gravimetrically by deducting the free monosaccharides originating from free SS, and they were absent. Total C and N concentration and related C:N ratio were similar among different temperature regimes, whereas starch-free C concentration was much higher at 8/6 ℃ than at the two warmer temperatures (Table 2-2).

Growth temperature also affected the concentration of the two main SC fractions in the bulb ($P < 0.01$ for both fractions, Table 2-1); the plants grown at the coolest temperature exhibited the highest concentrations. At all growth temperatures, the ratio of 'cellulose and lignin' fraction to hemicellulose fraction were always around 1.0 (Table 2-1). On a starch-free basis, in contrast, higher concentrations of hemicelluloses were observed in warmer-grown plants ($P = 0.021$), whereas the concentrations of 'cellulose and lignin' fraction did not significantly differ among growth temperatures ($P > 0.05$, Table 2-1). C:N ratio of the 'cellulose and lignin' fraction were higher than those from the total biomass and did not differ among the three growth temperatures ($P = 0.470$, Table 2-2).

Table 2-2. Total C and N concentrations (mg C or N. g DM^{-1}), related C:N ratio, and starch-free C concentration [mg C. g (starch-free DM) $^{-1}$] present at the end of growth period in the bulb of *E. americanum* grown at day/night temperatures of 8/6 ℃, 12/8 ℃, and 18/14 °C. C and N concentrations of 'cellulose and lignin' fraction (mg C or N. g $fraction^{-1}$) and related C:N ratio are also presented. All values represent mean ± standard error of the mean (SEM) (n = 5). Results of one-way ANOVAs (F values and P values) testing the effect of growth temperature are presented. Different letters in rows refer to significant differences (P < 0.05) among growth temperatures. Absence of letters in rows indicates non-significant differences.

	8 / 6 ℃	**12 / 8 ℃**	**18 / 14 ℃**	F value	*P* value
Bulb					
Total C (mg. g DM^{-1})	382.8 ± 1.5	386.8 ± 0.9	386.8 ± 1.6	2.66	*0.111*
Total N (mg. g DM^{-1})	10.8 ± 0.8	10.3 ± 0.2	11.3 ± 0.4	0.97	*0.407*
C : N ratio	36.3 ± 2.5	37.7 ± 0.7	34.3 ± 1.4	0.95	*0.414*
Starch-free C (mg. g DM^{-1})	109.1 ± 8.8 a	68.5 ± 11.9 b	45.9 ± 4.7 b	12.74	**0.001**
Cellulose and lignin fraction					
mg C. g $fraction^{-1}$	338.8 ± 9.4	351.5 ± 3.6	361.3 ± 1.4	3.74	*0.055*
mg N. g $fraction^{-1}$	7.3 ± 1.1	7.2 ± 0.5	6.6 ± 0.5	0.27	*0.768*
C : N ratio	48.8 ± 4.6	49.9 ± 3.6	55.5 ± 3.9	0.80	*0.470*

Table 2-3. Composition in monosaccharides of hemicelluloses from the cell walls of bulbs of *E. americanum* grown at day/night temperatures of 8/6 ℃, 12/8 ℃ and 18/14 ℃. The abundance of each monosaccharide is expressed as a percentage of the total hemicellulose pool. Values are means ± standard error of the mean (SEM) for each temperature regime (n = 5). Results of one-way ANOVAs (F values and *P* values) testing the effect of growth temperature are presented.

	8/6 ℃	**12/8 ℃**	**18/14 ℃**	F value	*P* value
Arabinose	35.7 ± 1.2	35.3 ± 2.3	35.3 ± 0.7	0.02	*0.981*
Xylose	30.3 ± 1.3	30.1 ± 1.8	31.3 ± 0.7	0.24	*0.789*
Galactose	20.4 ± 2.1	17.5 ± 1.2	20.9 ± 0.7	1.62	*0.238*
Glucose	9.1 ± 1.1	13.5 ± 5.5	8.7 ± 0.7	0.67	*0.529*
Fucose	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	0.61	*0.562*
Mannose	1.4 ± 0.1	1.0 ± 0.2	1.2 ± 0.1	2.44	*0.129*
Rhamnose	1.3 ± 0.3	0.7 ± 0.1	0.6 ± 0.1	2.71	*0.107*
Galacturonic acid	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	2.67	*0.110*
Glucuronic acid	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.63	*0.550*

2.3.3 Monosaccharides Composition of Hemicelluloses

The bulbs had a similar composition in monosaccharides within the hemicellulose fraction at the three growth temperatures ($P > 0.05$ for all monosaccharides, Table 2-3). Arabinose, xylose, galactose, and glucose were the major monosaccharides of the hemicellulose fraction, and accounted on average for 35.5 %, 30.6 %, 19.7 % and 10.5 %, respectively, of all monosaccharides. Fucose (1.5 %), mannose (1.2 %), rhamnose (0.9 %), galacturonic acid (0.4 %), and glucuronic acid (0.1 %) were minor components of the hemicellulose fraction.

2.3.4 Carbon Partitioning in the Starch-Free C

The contribution of C from free SS to the total amount of starch-free C of the bulb (C partitioning) significantly differed among the three growth temperatures ($P < 0.05$, Fig. 2-2), with higher contributions in the bulb of plants grown at the two warmer temperatures (16.1 and 11.7 % at 18/14 ℃ and 12/8 ℃, respectively *vs*. 7.2 % at 8/6 ℃). C partitioning to SC was significantly higher in warmest-grown plants ($P < 0.05$ for both structural fractions), where hemicellulose fraction accounted for 35.9 %, and 'cellulose and lignin' fraction accounted for 28.9 % to the total starch-free C amount of the bulb. At the coolest temperature, C investment into these two structural fractions was much lower, which accounted for 19.1 % and 16.0 % for the hemicellulose fraction and 'cellulose and lignin' fraction, respectively. The "remaining" fraction (total starch-free C amount of the bulb minus C amounts from free SS fraction and from structural fractions) accounted for about 57.6 %, 45.4 %, and 19.1 % of total starch-free C amounts in the bulb of plants grown at 8/6 ℃, 12/8 ℃, and 18/14 ℃, respectively ($P < 0.05$, Fig. 2-2).

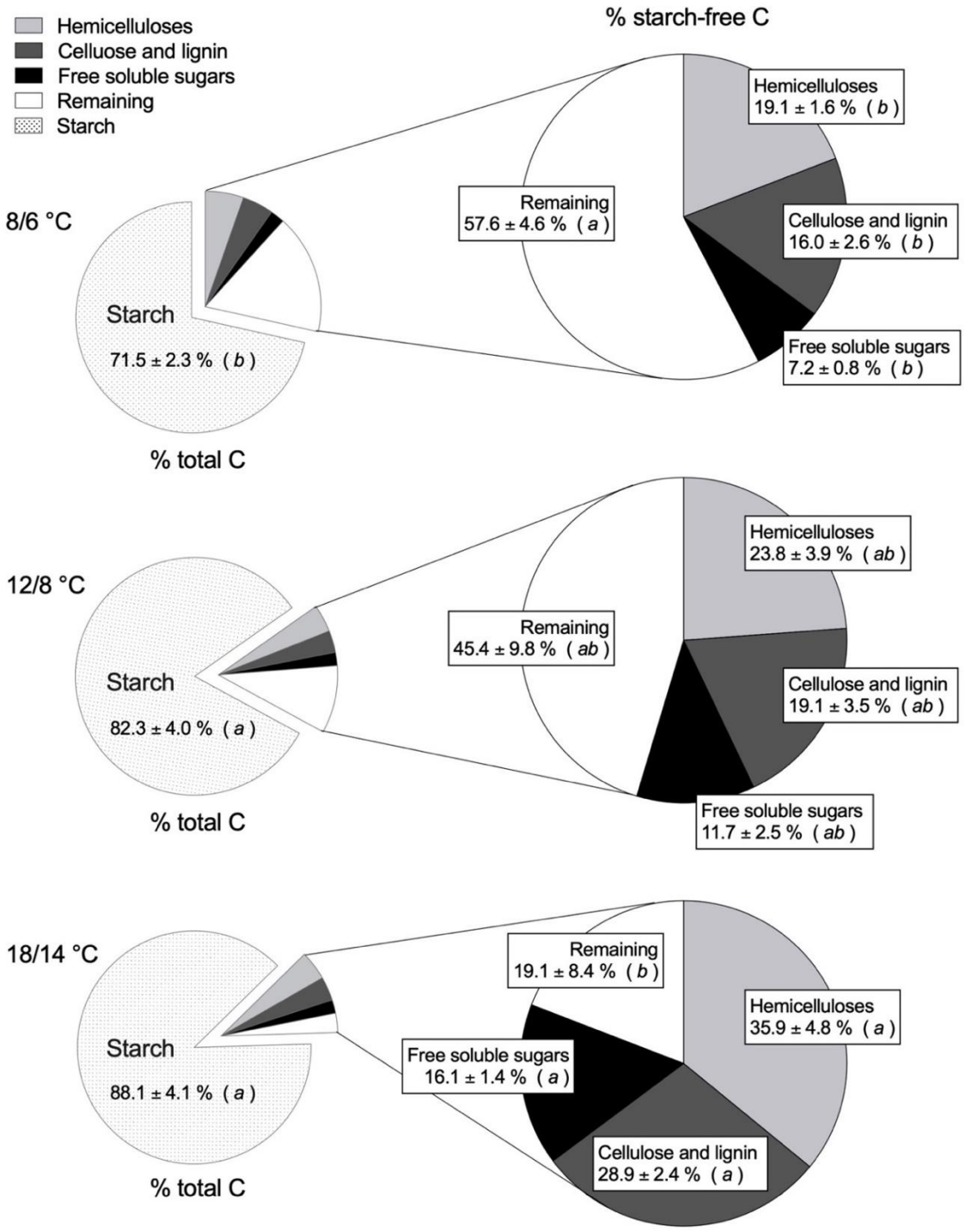


Figure 2-2. Amount of C in different purified fractions expressed in percent of the total C (left panels) and starch-free C amount (right panels) in the bulb of *E. americanum* grown at day/night temperatures of 8/6 ℃, 12/8 ℃ and 18/14 ℃. Values are means ± standard error of the mean (SEM) for each temperature regime (n = 5). Different letters refer to significant differences ($P < 0.05$) among growth temperatures.

2.4 Discussion

The final bulb biomass of *E. americanum* was influenced by growth temperature, with more biomass accumulating at cooler growth temperature compared to warmer temperature regimes. These results are consistent with previous observations in the same or similar geophyte species (Lapointe and Lerat, 2006; Badri *et al.*, 2007; Gandin *et al.*, 2011; Bernatchez and Lapointe, 2012). This response mainly reflects temperature effects on the new bulb growth. By contrast, comparable biomass of the mother bulb suggests that the remobilization of previously stored carbohydrates in the mother bulb was not affected by the growth temperature. In *E. americanum*, a higher bulb size at cool temperature was tentatively explained by an improved equilibrium between C fixation capacity and sink strength (Gandin *et al.*, 2011; Dong *et al.*, 2018). This strength is known to be driven by sucrose breakdown (supported by invertase and by sucrose synthase), which largely contributes to providing assimilated C for sink metabolism (Sturm and Tang, 1999; Asano *et al.*, 2002; Koch, 2004; Ruan, 2014). Overexpression and antisense inhibition of sucrose synthase in tubers of potato plants (Zrenner *et al.*, 1995), in carrot plants (Tang and Sturm, 1999), and in cotton seeds (Ruan *et al.*, 2003) have confirmed its crucial role in sink strength and C partitioning (Koch and Zeng, 2002; Koch, 2004; Ruan, 2014). In *E. americanum*, Gandin *et al.* (2011) suggested that the bulb cell maturation was delayed at cooler temperatures because of delayed activation of sucrose synthase, which led to an extended cell expansion which in turn allowed for a higher sink capacity than at warmer temperatures. As sucrose synthase was shown to be a key enzyme providing substrates for biosynthesis of starch (Zrenner *et al.*, 1995), cellulose, and hemicelluloses (Amor *et al.*, 1995; Chourey *et al.*, 1998), modulation of its activity in response to growth temperature treatment could result in modified C partitioning.

Starch is the dominant carbohydrate in the bulb of *E. americanum*. This insoluble and osmotically inert compound is considered a long-term C storage for many different organs, essential for resprouting under favorable climatic conditions. The amount of starch is controlled primarily by sucrose transport from source to sink tissues and its conversion to starch. Starch biosynthesis involves the activity of four enzymes, with AGPase (ADP-glucose pyrophosphorylase) catalyzing the first step (Thitisaksakul *et al.*, 2012). Gandin *et*

al. (2011) showed that, in the bulb of *E. americanum*, the activity of AGPase increased during the first few days to reach maximal catalytic activity early in the season, with slower initial rate at cool temperature (8 ℃) compared to warmer temperature (18 ℃). Numerous recent studies have well characterized this biosynthetic pathway, especially in cereal grains (Jeon *et al.*, 2010; Keeling and Myers, 2010; Thitisaksakul *et al.*, 2012; Kumar *et al.*, 2018). Low temperature was shown to slow but also to extend starch biosynthesis, so that starch content of cereal grains was unaltered (Ahmed *et al.*, 2008; Thitisaksakul *et al.*, 2012). These results agree with delayed kinetics of starch deposition observed in the bulb of *E. americanum*. By contrast, high temperature during the grain-filling period increases the grain dry matter accumulation rate but shorten the grain-filling period (Ahmed *et al.*, 2015). It has been observed in wheat that for every 1 ℃ increase in mean temperature, the duration of grain filling is shortened by approximately 3 days (ranged temperatures from 16 to 28 ℃) (Wiegand and Cuellar, 1981), which results in reduction of grain weight. These results also agree with our data meaning that the activity in different sinks and in particular starch accumulation is regulated by temperature.

In the present study, NSC composition of the final bulb of *E. americanum* was comparable to that in the corm of *Crocus vernus* (Ranwala and Miller, 2008). Starch was the major NSC detected, whereas free soluble sugars (mainly sucrose) were present at lower concentrations, and fructans and glucomannans were not detected. Comparable NSC composition was previously observed in the bulbs of *Lilium longiflorum* (Miller and Langhans, 1990) and *Galanthus nivalis* (Orthen and Wehrmeyer, 2004). By contrast, the bulb of *Allium* species accumulate high levels of fructans and no starch, while the bulb of *Iris* or *Narcissus* accumulates both starch and fructans (Ranwala and Miller, 2008). The same diversity was observed by Orthen (2001b) in a survey of polysaccharides in storage organs of 63 geophytes species native to the winter-rainfall region of South Africa. The author assumed that these two carbohydrates fulfilled different roles during specific phenological phases. For geophytes with similar fructans and starch contents, it is proposed that a differential use of these two NSC would ensure that some C remains available under adverse conditions (Orthen, 2001a). Such assumption cannot be verified in our study as NSC composition was characterized at a single physiological stage. Glucomannans occur

much less frequently than fructans as a reserve carbohydrate in geophytes (Miller, 1992; Ranwala and Miller, 2008), and the species that contain glucomannans as the dominant storage seemed to have a low-level of starch or no starch (Ranwala and Miller, 2008; Gille *et al.*, 2011). Therefore, the absence of glucomannans seems reasonable in this starch-rich bulb.

Starch deposition was found to occur synchronously with the increase in bulb cell size in *E. americanum* (Gandin *et al.*, 2011). However, starch accumulation could not fully explain the accumulation of C in the bulb, as higher starch-free C concentrations (on DM basis) were observed in the bulb of cool-grown plants compared to the other treatments (Table 2-2). Modifications of C partitioning into the cell wall components were suspected. Through $^{14}CO_2$ labelling, Lundmark *et al.* (2009) showed that, in the corm of *C. vernus*, recently assimilated ^{14}C was partitioned into NSC (up to 90 % of the total amount of ^{14}C in the corm) but also into the cell wall fraction (less than 10 %). In this previous study, the "cell wall fraction" corresponded to the final insoluble fraction resulting from their purification procedure. Here, we developed a method to analyze the qualitative modifications of the structural C pools in *E. americanum* bulbs grown at different growth temperatures. Due to the low biomass of individual bulbs, it was not possible to separate all the components of the cell walls completely, and we collected two fractions: hemicelluloses and 'cellulose and lignin' fractions. To validate the method, we compared our data, expressed on a DM basis, with those from the literature: for both fractions, our concentrations were lower than those previously reported in the literature (Hoch, 2007; Pauly and Keegstra, 2008; Vogel, 2008; Schädel *et al.*, 2009), and those observed by Schädel *et al.* (2010a) for different plant functional types. These differences could be partially due to the fact that cell walls differ substantially among plant functional types (Pauly and Keegstra, 2008), but is most likely also explained by the diluting effect caused by the high starch concentrations. Indeed, whatever the growth temperature, SC concentrations expressed on a starch-free DM basis were closer (16 to 28 % starch-free DM) to those presented by Schädel *et al.* (2010a), and the ratios of 'cellulose and lignin' fraction to hemicelluloses were also consistent with those obtained by Schädel *et al.* (2010a) for non-woody tissues. Moreover, the higher C:N ratio for the 'cellulose and

lignin' fraction compared to that of the initial biomass confirms that this fraction contained carbon-rich components, although nitrogenous compounds such as structural proteins remain present (around 7 mg. g^{-1} fraction, which represented only 0.3 mg. g DM^{-1}, data not shown).

Phylogenetic variations are often observed in the proportion of SC represented by hemicelluloses, with generally less than 30 % of the total cell wall pool, and often somewhat higher percent in monocots than in dicots (Schädel *et al.*, 2010a). In the bulb of *E. americanum*, the abundance of hemicelluloses within total SC pool was fairly high (more than 50 %), whereas much lower proportions were observed in other bulbous or tuberous plants, for instance, nearly 40 % in bulbs of onions (*Allium cepa*) (Mankarios *et al.*, 1980), and even lower in tubers of cassava (*Manihot esculenta*), jicama (*Pachyrrhizus erosus*), potato (*Solanum tuberosum*), and sweetpotato (*Ipomoea batatas*) (Klockeman *et al.*, 1991; Salvador *et al.*, 2000). This diversity might be at least partially due to methodological differences, but nevertheless their remarkable abundance in the bulb of *E. americanum* suggests an additional function for hemicelluloses other than purely structural support.

As the main type of hemicelluloses in the bulb of *E. americanum*, arabinoxylans also represent the major hemicellulose components in similar organs, such as jicama tubers (*Pachyrrhizus erosus*) and Chinese water chestnut corms (*Eleocharis dulicis*) (Klockeman *et al.*, 1991), and in other tissues or species, like starchy endosperm of cereal grain and grasses (Ebringerova *et al.*, 2005; Pauly and Keegstra, 2008; Schädel *et al.*, 2010a). The functional properties of arabinoxylans, especially in cereals, in water-holding capacity and protein foam stabilization make them highly interesting in the food industry (Muralikrishna and Rao, 2007). However, to our knowledge, their physiological functions in reserve organs have not yet been characterised. The similar abundance of each hemicellulose-derived monosaccharides investigated in the bulb of *E. americanum* regardless of growth temperature agrees with previous findings showing that characteristic pattern of monosaccharides for each functional plant group did not change with variation in source activity (Ebringerova *et al.*, 2005; Schädel *et al.*, 2010b).

Carbon partitioning into each fraction investigated in the present study differed significantly among temperature regimes. On a starch-free basis, the reduced C partitioning into free SS fraction in cooler-grown plants supported previous results where a reduced starch-free amount of SS in the bulb of *E. americanum* has been reported under cooler condition (Gandin *et al.*, 2011; Dong *et al.*, 2018), which would help maintain sink activity for a much longer period. Lower assimilation rates in cooler-grown plants combined with a slightly higher SS amount in leaves of plants grown at cooler temperature (Dong *et al.*, 2018), most likely decreased the amount of C available for translocation to the sink, which in turn could translate into a reduction in the amount of C invested into the SS fraction within the bulb. The free SS fraction only contained sucrose, with essentially no glucose or fructose; this could be explained by the early decrease of invertase activity (Gandin *et al.*, 2011). The contributions of this enzyme have been shown to occur early on, i.e. during initiation and cell expansion within the bulb (Koch, 2004; Gandin *et al.*, 2011), leading to an early decline of hexoses.

Unlike the free SS fraction, C invested in the "remaining" fraction was considerably higher at cool temperature than at warmer temperature [20 % DM (17 % of total C, and more than 50 % of starch-free C) at 8/6 ℃ *vs.* 6 % DM (3 % of total C, and less than 20 % of starch-free C) at 18/14 ℃]. Lipid and nitrogen content (including amino acids and proteins) are two other main fractions not quantified here. Generally, roots and tuber crops contain low amount of lipids, typically between 0.5 and 0.8 % DM (Mondy and Mueller, 1977; Bonnier *et al.*, 1997; Prescha *et al.*, 2001; Lebot *et al.*, 2004; Lewu *et al.*, 2010). Through the data estimated from other extraction experiments, the amounts of C invested in amino acids and proteins in the bulb of *E. americanum* were also limited to about 3−4 % DM (Dong et al., unpublished data). Lipids, proteins and amino acids most likely do not account for all C investment in the "remaining" fraction at the two cooler growth temperatures. The assay method of SS and starch in the present study was not highly specific, likely leading to a relative inaccuracy in the proportions calculated, but there must be some other compounds, which were beyond the initial scope of the present study and were most likely lost or ignored in the extraction process. We posit that the

unexpected fraction may correspond to pectins. As the most complex cell wall components, pectins have major structural and functional impacts on cell wall characteristics and growth control (Peaucelle *et al.*, 2012; Wolf and Greiner, 2012). It has been demonstrated that pectin concentrations varied between 15 and 30 % DM in certain storage tissues, such as sugar beet and potato (Turquois *et al.*, 1999; Levigne *et al.*, 2002) and their proportions in the cell wall could reach up to 50 % cell wall components (Klockeman *et al.*, 1991; Salvador *et al.*, 2000; Wolf and Greiner, 2012). Their role as storage compounds has already been reported in the cotyledons of certain lupin species and in Arabidopsis seeds (Crawshaw and Reid, 1984; Gomez *et al.*, 2009). They are involved in the temperature-dependent modifications of the cell wall structure in various species, such as pea seedlings and oilseed rape plants, reflecting the plant cold-acclimation strategy (Weiser *et al.*, 1990; Solecka *et al.*, 2008; Baldwin *et al.*, 2014). It has also been shown in leaves of winter oilseed rape that low temperature increased pectin levels in the cell wall, and modified the composition in pectic monosaccharides, whereas the proportions of hemicelluloses and cellulose in the cell wall remained unaffected (Kubacka-Zębalska and Kacperska, 1999). To the best of our knowledge, changes of pectins and their monosaccharides in response to growth temperature have not been reported in bulbous species. Their possible role in the response to cool temperature in the bulb of *E. americanum* deserve further experiments and investigation.

The greater C partitioning into structural pools at warmer temperature, both in the hemicellulose and in the 'cellulose and lignin' fractions, brings support to previous findings in *C. vernus* that more C is invested in the cell wall material at the end of the season under warmer temperature than under cool temperature regimes (Lundmark *et al.*, 2009). But it is noteworthy that the different cell wall components were not separately distinguished in their analysis. Higher temperature stimulates C acquisition by assimilation which should increases availability of translocated C to the sink (Gandin *et al.*, 2011; Dong *et al.*, 2018). As a result, the bulb cells reached their final size more rapidly and accumulate starch more rapidly at warmer than at cooler temperatures (Gandin *et al.*, 2011). Once cells are filled with starch, the capacity to accumulate C in cell wall components should help plants reduce sink limitations at this higher temperature, and thus improve the source−sink balance at

least for a few days. The higher and faster increase of sucrose synthase activity previously reported in the bulb of *E. americanum* grown at warmer temperature (Gandin *et al.*, 2011), indicative of a shift from cell elongation to cell maturation, may support this opinion. This enzyme has been well demonstrated to contribute to starch and cellulose biosynthesis (Barratt *et al.*, 2009; Baroja-Fernández *et al.*, 2012; Braun *et al.*, 2014). Timing of its contribution fits the adjustment of C partitioning between NSC and SC fractions in the bulb. However, different C destinations in cell wall components would result in substantially different impacts on plant growth or on C budget.

A greater investment of C in 'cellulose and lignin' fraction, especially in lignin, may exert harmful influences on plant growth. Lignin is often low in abundance in the cell wall, for example, less than half of the cellulose fraction in switchgrass (Pauly and Keegstra 2008). It has been shown that lignin does not occur strictly in tissues with secondary walls, and numerous studies detected the deposition of lignin in the primary walls of monocots, such as *Agave sisalana*, *Miscanthus*, and coleoptiles of *Zea mays* L. (Müsel et al. 1997, Del Río et al. 2007, Novo-Uzal et al. 2012, Pang et al. 2018). Lignin synthesis is costly, which could consequently imply C losses through respiration and thus a strong influence on the C budget (Poorter and Villar 1997). Following this assumption, we found that bulb respiration in *E. americanum* was stimulated as sink limitation built up at warmer temperatures (Dong et al. 2018), and was tightly correlated with sucrose synthase activity (Gandin et al. 2009). Two enzymes involved in lignin biosynthesis (peroxidase and laccase) have been found to exhibit high activities in response to high temperature in *Agrostis* grass species and strawberry plants (Gulen and Eris 2004, Xu et al. 2008); whereas most other enzymes increased in activity at cold temperature, for instance, in sweet potato and *Phaseolus vulgaris* seeds (Kim et al. 2013, Badowiec and Weidner 2014, Le Gall et al. 2015). Therefore, results from the literature do not fully support the hypothesis of a greater investment into lignin at warmer temperatures in *E. americanum* bulbs. However, this assumption could partially explain the shorter duration of growth in warmer-grown plants than those grown at cooler temperature, since lignin deposition would strongly limit further cell elongation (Müsel et al. 1997).

Unlike cellulose and lignin, greater C partitioning into hemicelluloses of cell wall at warmer temperature could help improve the source−sink balance. There are already many evidences that hemicelluloses in cell walls may be recycled and used during periods of reduced C supply from photosynthesis (Hoch, 2007; Schädel *et al.*, 2009). The reserve function of hemicelluloses has also been well demonstrated in needles of some conifers and in branch sapwood of deciduous tree species (Robakidze and Bobkova, 2003; Hoch, 2007; Schädel *et al.*, 2009). Knowing that parenchyma cells in bulbs or corms have very thin cell walls that collapse in the following spring, it is therefore more likely that the investment of C in cell wall components at the end of season would occur in a reserve form, i.e. hemicelluloses, rather than in cellulose or lignin. We expect that the investment of C in cell wall components at the end of the season would occur in a reserve form, i.e. hemicelluloses, rather than in cellulose or lignin. However, the remobilization and reserve function of hemicelluloses besides their primarily structural function in *E. americanum* needs more in-depth analysis, for example, by using ^{13}C-labeling experiments.

In conclusion, enhanced bulb biomass accumulation observed at cool growth temperature was primarily reflected into the new bulb growth. We confirmed that the main reserve within the bulb of *E. americanum* was starch, and that fructans and glucomannans were absent. At cool temperature, reduced C partitioning into starch-free soluble sugars in the bulb may be related to the contributions of sucrose-cleaving enzymes along with a longer growth period. At warmer temperature, greater C partitioning into cell wall components appears as an efficient way to minimize sink limitations once the cells are filled with starch. However, investing more C in the 'cellulose and lignin' fraction could have harmful consequences on plant growth and affect C budget, whereas increased C partitioning into cell wall hemicelluloses seems to be better adapted to source–sink equilibrium because of its potential role as a C reserve. Further in-depth studies will be required, on the one hand, to verify the potential role of hemicelluloses as reserves in the bulb of *E. americanum*, and on the other hand, to determine the possible role pectins might play in the response to cool temperature.

2.5 Acknowledgements and Funding

This research was financially supported by a Discovery grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to LL. The authors would like to thank SILVATECH from UMR 1434 SILVA, 1136 IAM, 1138 BEF and 4370 EA LERMAB EEF research center INRA Nancy-Lorraine for its contribution to C and N microanalysis. SILVATECH facility is supported by the French National Research Agency through the Laboratory of Excellence ARBRE (ANR-11-LABX-0002-01). The authors gratefully acknowledge François Larochelle for his expert technical assistance in growth chamber experiments. The authors also sincerely thank all colleagues in the laboratory who participated to plant harvests: Pierre-Paul Dion, Jade Boulanger-Pelletier and Karolane Pitre.

Chapitre 3. Hormonal and metabolic profiling during leaf aging and senescence suggests that some specific sugars might trigger leaf senescence in the spring ephemeral *Erythronium americanum*

Yanwen Dong[1,2], Dominique Gérant[2], Sergi Munné-Bosch[3], Maren Müller[3] and Line Lapointe[1]

[1] Département de Biologie and Centre d'Étude de la Forêt, Université Laval, Québec, QC, Canada

[2] Unité Mixte de Recherches 1434 SILVA, Université de Lorraine/INRA/AgroParisTech, Faculté des Sciences et Technologies, 54506 Vandœuvre-lès-Nancy cedex, France

[3] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona, Facultat de Biologia, Avinguda Diagonal 643, 08028 Barcelona, Spain

Résumé

De nombreux phytohormones et métabolites sont connus pour jouer des rôles clés dans la régulation du développement des plantes et la sénescence. Des études antérieures ont suggéré que la sénescence foliaire chez les éphémères printanières était induite par une réduction de l'activité du puits une fois la croissance de l'organe pérenne terminée. Nous avons caractérisé les profils phytohormonaux et métabolomiques dans les feuilles et les bulbes d'*Erythronium americanum* cultivés suivant trois températures de croissance afin d'identifier le signal déclencheur potentiel impliqué dans l'induction de la sénescence. Des profils phytohormonaux et métabolomiques distincts caractérisaient les différents stades de développement de la feuille et du bulbe. Une augmentation transitoire des sucres phosphorylés en début de saison pourrait refléter un déséquilibre source−puits momentané alors que la photosynthèse des feuilles est élevée, mais que le nouveau bulbe est encore petit. Par la suite, de nombreux sucres et polyols ont augmenté en abondance dans les feuilles, et leurs effets ont été très probablement contrecarrés par les concentrations accrues de cytokinines, qui sont bien connus pour retarder la sénescence foliaire. Au début de la sénescence, l'acide 1-aminocyclopropane-1-carboxylique, l'acide abscissique et l'acide salicylique ont augmenté, mais seulement aux températures de croissance plus élevées. Certains sucres, à savoir le 2-O-glycérol-β-D-galactopyranoside, le mannose, le fructose, le sorbose et le maltose, pourraient être les facteurs de signalisation les plus probables induisant la sénescence foliaire, et en particulier le 2-O-glycérol-β-D-galactopyranoside et le maltose, qui ont présenté des modifications concomitantes dans le bulbe et la feuille vers la fin de la saison.

Mots-clés: *Erythronium americanum*, sénescence foliaire, profil phytohormonal, métabolomique

Abstract

Many phytohormones and metabolites are known to play key roles in regulating plant development and senescence. Previous studies suggest that leaf senescence in spring ephemerals is induced by a reduction in sink activity once the growth of the perennial organ is completed. We characterised the phytohormonal and metabolomic profiles in the leaves and bulbs of *Erythronium americanum* grown at three growth temperatures, in order to identify potential triggering signal involved in inducing leaf senescence. Distinct phytohormonal and metabolomic profiles characterised the different developmental stages of both the leaf and the bulb. Transient increase of phosphorylated sugars in early season might reflect a momentary source–sink imbalance while leaf photosynthesis is high but the new bulb is still small. Later on, many sugars and sugar alcohols increased in abundance in the leaves, and their effects were most likely counteracted by the increasing concentrations of cytokinins, which are well-known to delay leaf senescence. At the onset of senescence, 1-aminocyclopropane-1-carboxylic acid, abscisic acid and salicylic acid increased but only under warmer growth condition. Some sugars, namely 2-O-glycerol-β-D-galactopyranoside, mannose, fructose, sorbose, and maltose, might be the most likely signalling factors inducing leaf senescence, and in particular 2-O-glycerol-β-D-galactopyranoside and maltose, which exhibited concomitant changes in the bulb and the leaf towards the end of the season.

Keywords: *Erythronium americanum*, leaf senescence, phytohormonal profiling, metabolomics

3.1 Introduction

The spring ephemeral *Erythronium americanum* Ker-Gawl. (Liliaceae), or trout lily, is an herbaceous geophyte that is common to temperate North American hardwood forests. During spring, this species rapidly accumulates carbohydrates in the perennial organ (bulb), after which the leaves start to senesce. This response suggests that leaf senescence is induced by sink limitation (Lapointe, 2001). To test this assumption, source–sink relationships were modulated especially by growing plants under different temperature regimes. At cooler temperatures, the new bulb grows for a much longer period of time, leading to a larger bulb and to delayed leaf senescence (Badri *et al.*, 2007; Gandin *et al.*, 2011; Bernatchez and Lapointe, 2012; Dong *et al.*, 2018). These results support the hypothesis that leaf senescence is induced by a sink limitation in spring ephemerals, but the mechanisms that are involved in the induction of leaf senescence have not been fully characterised.

Senescence is tightly controlled by phytohormones and by other metabolites. Yet, it is also strongly influenced by various environmental factors including high or low temperature, drought, ozone, nutrient deficiency, and pathogen infections, amongst others (Wolters and Jürgens, 2009; Taiz and Zeiger, 2010). Our current understanding of the relationship between environmental factors and leaf senescence stems mainly from the study of senescence responses to phytohormones, such as abscisic acid (ABA), jasmonic acid (JA), ethylene, and salicylic acid (SA), which are widely involved in plant responses to various biotic and abiotic stresses. These stresses affect the synthesis or hormonal signalling pathways that eventually trigger the expression of stress-sensitive genes, which in turn can induce leaf senescence (Lim *et al.*, 2007). In addition to stress-related phytohormones, cytokinins (CKs), e.g. *trans*-zeatin (Z), are involved in the regulation of leaf senescence (Letham *et al.*, 2003). Indeed, many phytohormones clearly play a central role in regulating the onset and progression of senescence by acting at various levels (physiological, metabolic and molecular) (Arrom and Munné-Bosch, 2012).

The potential role played by different metabolites in the leaf senescence process has also been the subject of various studies. For instance, glucose and fructose were shown to

accumulate prior to the first visible signs of senescence in the shoots of *Galanthus nivalis* L. (common snowdrop), which is a spring geophyte (Orthen and Wehrmeyer, 2004), or before the decrease in chlorophyll concentrations in the leaves of *Arabidopsis* (Diaz *et al.*, 2005). These responses suggest that sink limitation is the driving force inducing leaf senescence in these cases. In leaves of cultivated tobacco (*Nicotiana tabacum* L.), a decline in photosynthetic function was accompanied by concomitant accumulation of sugars; indeed, sugars may be important signalling molecules during leaf senescence (Wingler *et al.*, 1998; Masclaux *et al.*, 2000). Glutamate and aspartate were the most abundant amino acids in young *Arabidopsis* leaves, while minor amino acids like tyrosine, leucine, isoleucine and GABA (γ-aminobutyric acid) dramatically accumulated with leaf aging (Diaz *et al.*, 2008). Tocopherols, in cooperation with other antioxidants, such as ascorbic acid, are well known for their major role in protecting plants against oxidative stress, which typically occurs during leaf senescence (Munné-Bosch *et al.*, 1999; Keleş and Öncel, 2002; Munné-Bosch, 2005). Phytohormones, sugars, amino acids and antioxidants thus involve multiple metabolic pathways that respond to various internal and external factors to establish a complex network of senescence-regulating pathways (Lim *et al.*, 2007).

In geophytes, specific phytohormone and metabolite changes occur within the storage organ throughout the season (De Hertogh and Le Nard, 1993), with some being potentially associated with leaf senescence induction. Correlations between high GA levels and bud sprouting, as well as reductions in ABA contents and dormancy have been reported in diverse geophytes (Yamazaki *et al.*, 2002; Suttle, 2004; Naor *et al.*, 2008). Accumulation of starch and the production of storage proteins are the main biochemical changes that take place during tuber initiation in potatoes (*Solanum tuberosum* L.) (Visser *et al.*, 1994). Several carbohydrates (primarily sucrose) play an important role in dormancy initiation (Claassens, 2002). A signalling sink-to-source metabolic flux originating from sink organs likely exists, which could trigger the induction of leaf senescence, but none has been proposed so far to our knowledge.

Until recently, phytohormones and other metabolites that are involved in the induction of senescence were investigated mostly on an individual basis. The signalling

pathway that induces leaf senescence has only been partially uncovered, and it appears to vary depending upon the factor driving senescence (stress, phenology or sink limitation). New technological approaches such as metabolomics allow to investigate the potential role of a much larger pool of metabolites in the induction of leaf senescence (Roessner *et al.*, 2000; Diaz *et al.*, 2005). Furthermore, the analysis of dynamic changes in endogenous concentrations of major phytohormones (hormonal profiling) using ultra-performance liquid chromatography mass spectrometry (UPLC/ESI-MS/MS) may be a very useful complement to metabolomic studies (Müller and Munné-Bosch, 2011).

The present study characterised phytohormonal and metabolomic profiles in the leaves and bulbs of *E. americanum* in order to identify signalling molecules that lead to leaf senescence under sink-limited conditions. We posit that certain specific sugars would either accumulate or strongly decrease a few days prior to the first visual sign of leaf senescence, thereby inducing the synthesis of phytohormones (i.e., ethylene, ABA, SA or JA), which would then induce leaf senescence. Source–sink relationships were modulated by using different growth temperature regimes (day/night: 8/6 °C, 12/8 °C, and 18/14 °C); subsequent analyses were performed throughout the growth season at both leaf and bulb level. This approach distinguished a general phytohormonal and metabolomic profile that was associated with the induction of leaf senescence from the individual profiles that are temperature-specific. To complement the metabolomics data, which are semi-quantitative, we quantified total amino acid, soluble sugars and total soluble protein concentrations. Finally, leaf protease activities were also measured to confirm that the visual signal of leaf senescence corresponded to the actual induction of nitrogen remobilisation.

3.2 Material and Methods

3.2.1 Plant Material, Growth Conditions and Sampling

Erythronium americanum bulbs were collected in April 2011 from a sugar maple forest that was located in Saint-Augustin-de-Desmaures, nearby Quebec City (QC, Canada), as shoots were emerging from the soil. Bulbs of similar diameter (6 – 8 mm) were selected and planted individually in plastic pots containing Turface (calcined clay granules, Applied

Industrial Materials Corp., Buffalo Grove, IL, USA) as substrate, then randomly assigned to each of three growth chambers (PGW36, Conviron Inc., Winnipeg, MB, Canada) under a photon flux density (PPFD) of 300 μmol. m^{-2}. s^{-1} for a 14h photoperiod. Another series of plants were harvested in early spring 2012 while shoots were sprouting and immediately potted and placed in growth chambers under the same conditions as those harvested in April 2011. Temperature regimes in the two experiments were based upon those employed in a study by Gandin *et al.* (2011). Three growth temperature regimes were applied in the experiments: 8/6 °C (day/night), 12/8 °C, and 18/14 °C, with respective relative humidities (RH) of 50 %, 65 %, and 75 %. RH was modulated as a function of temperature to maintain a constant vapour pressure deficit (VPD) amongst growth chambers. Plants were watered regularly and fertilised weekly with 10 % Hoagland's solution for optimal growth (Lapointe and Lerat, 2006).

Plants were harvested following specific phenological stages as described in Gandin *et al.* (2011). Briefly, the harvests were performed at the beginning of the experiment (T1; day 0, when plants were moved to the growth chambers, common to all temperature regimes), at initiation of leaf unfolding (T2; day 3, 4 and 5 at 18/14 ℃, 12/8 ℃ and 8/6 ℃, respectively), at complete leaf unfolding (T3; day 5, 7 and 9 at 18/14 ℃, 12/8 ℃ and 8/6 ℃, respectively), at first visual sign of leaf senescence (T8; day 17, 21 and 29 at 18/14 ℃, 12/8 ℃ and 8/6 ℃, respectively), and at complete leaf senescence (T9; day 27, 35 and 44 at 18/14 ℃, 12/8 ℃ and 8/6 ℃, respectively). Between complete leaf unfolding (T3) and the initiation of leaf senescence (T8), harvesting was staggered amongst the three temperature regimes, i.e., every 2 days for 18/14 °C, 3 days for 12/8 °C, and 4 days for 8/6 °C. Although harvests were based upon leaf phenological stages, specific phenological stages could be identified for the bulbs. T1 to T3 corresponds to a period when the bulb acts as a sink and actively translocates carbon and nutrients to the leaf. Between T3 and T4, the old bulb continues to shrink and the new bulb is initiated. T5 to T7 corresponds to the growth and starch accumulation of the new bulb. At T8, the new bulb has reached its final size and, at T9, it has entered dormancy. As leaf and bulb studies were performed separately, their growth curves differed slightly; we had to label the 5^{th} harvest in the bulb study, T5/T6, because the total number of harvests for the bulb study was less than that in

the leaf study. Furthermore, metabolites and phytohormones at T9 were only analysed for the bulbs, since the strongly reduced mass of completely senesced leaves could lead to overestimating the abundance of the study compounds. At each harvest, plants were immediately frozen in liquid nitrogen, then transferred to a -80 °C freezer. Individual leaves and bulbs were lyophilised for 48 hours before analysis of proteolytic activities, phytohormones, biochemical (non-structural compounds), and metabolomic analysis.

3.2.2 Non-Structural Compounds

Non-structural compounds were extracted from 10 mg of leaf or bulb dry material with 650 µL of methanol/chloroform/water (12:5:3, v/v), after which the extracts were centrifuged at 17 000 g for 10 min at 4 °C. This second step was repeated twice and the resulting supernatants were pooled. Total amino acid concentrations were determined on the soluble fraction by colorimetry, as described by Yemm *et al.* (1955). Soluble sugar concentrations were determined colorimetrically, as described by Hansen and Møller (1975). All concentrations were reported on a dry-mass (DM) basis. For the determination of total amino acid concentrations, a 100 µL aliquot of the soluble fraction was mixed with 275 µL of citrate buffer (0.4 M, pH 5.25) and 300 µL of ninhydrin solution, and heated at 100 °C for 20 minutes. After cooling in an ice bath, 750 µL of 60 % (v/v) ethanol was added to this mixture. Absorbances (A_{570}) were read on a UV-visible spectrophotometer (DU 640 B, Beckman Coulter, Brea, CA, USA) and compared with a standard (leucine) curve. For the determination of soluble sugar concentrations, an aliquot of 100 µL of the soluble fraction was mixed with 1.5 mL of anthrone, incubated at 60 °C for 20 minutes, then cooled to room temperature. Soluble sugar concentrations were determined colorimetrically (A_{620}), with glucose used as the standard.

3.2.3 Soluble Proteins and Proteolytic Activities

Lyophilised leaves (15 mg DM) were ground in mortar and soluble proteins were extracted with 300 µL 100 mM HEPES-KOH buffer pH 7.5 containing 5 mM $MgCl_2$, 2 mM dithiothreitol and 2 % polyvinylpolypyrrolidone (PVPP). Following centrifugation at 20 000 g for 25 minutes, the supernatant was collected and total soluble protein concentrations

were determined according to Bradford (1976), using bovine serum albumin (BSA) as a standard. The remaining extracts were kept at -80 °C until use. Proteolytic activities were monitored kinetically in the leaf soluble extracts as changes in relative fluorescence units resulting from hydrolysis of synthetic fluorogenic substrates (Peptides International: www.pepnet.com). We used z-Arg-Arg-methylcoumarin (MCA) for cathepsin B-like proteases, MOCAc-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH_2 for A1 Cathepsin D/E-like proteases, z-Arg-methylcoumarin (MCA) for Trypsin-like proteases, and Glt-Ala-Ala-Phe-methylcoumarin (MCA) for chymotrypsin-like proteases. Enzyme activities were assayed at room temperature with leaf extracts (12.5 ng μL^{-1}) in pH 6.5 phosphate buffer for cathepsin B-like, in pH 3.0 citrate-phosphate buffer for cathepsin D/E-like, and in pH 7.5 phosphate buffer for both trypsin- and chymotrypsin-like proteases. Proteolysis was measured using a Fluostar Galaxy microplate fluorimeter (BMG; www.bmglabtech.com), with respective excitation and emission filters of 360 and 450 nm for the MCA substrate, or at 340 and 400 nm, respectively, for the MOCAs substrate.

3.2.4 Phytohormone Analysis

Lyophilised leaves and bulbs (100 mg DM) were ground in a MM400 mixer mill (Retsch GmbH, Haan, Germany) and extracted with 0.2 mL of methanol: isopropanol: glacial acetic acid (20:79:1; v/v/v) using ultrasonication (4–7 °C). The labelled forms of compounds (purchased from Olchemim LTD, Olomouc, Czech Republic) d_6-ABA, d_4-ACC, d_4-SA, d_2-GA_1, d_2-GA_4, d_2-GA_9, d_2-GA_{19}, d_2-GA_{20}, d_2-GA_{24}, d_5-IAA, d_6-2iP, d_6-IPA, d_5-Z, and d_5-ZR were added as internal standards. D_5-Z and d_5-ZR were used as internal standards for DHZ and DHZR, respectively. Following centrifugation (14 000 g for 15 min at 4 °C), the supernatant was collected and the pellet was re-extracted twice with 0.10 mL of the extraction solvent. Supernatants were then combined, centrifuged (14 000 g for 5 min at 4 °C) and filtered through a 0.22 µm PTFE filter (Waters, Milford, MA, USA). Samples were then analysed by UPLC/ESI-MS/MS and quantified according to Müller and Munné-Bosch (2011).

3.2.5 Metabolomic Analysis

3.2.5.1 Metabolite Extraction and Derivatisation

Leaf and bulb metabolites were extracted following the method described by Roessner *et al.* (2000). About 15 mg of ground powder from lyophilised samples were weighed into 1.5 mL reaction tube and mixed well with 1 mL of 100 % methanol for the extraction. Ribitol was added as an internal standard (50 µL of 2 g. L^{-1} H_2O) to normalise values from injection losses or other artefacts during sample preparation and the gas chromatography-mass spectrometry (GC-MS) run. The solution was incubated for 15 min at 70 °C (vortexing every 3 min), followed by addition of 1 mL of water. The mixture was centrifuged at 5 000 g for 10 min, followed by a drying in a SpeedVac vacuum concentrator without heating. Two-step derivatisation of the extracted metabolites was performed in 80 µL of methoxyamine hydrochloride (20 g. L^{-1} of pyridine) for 90 min at 30 °C, followed by 80 µL of MSTFA [*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide, ≥ 98.5 % (GC), Sigma-Aldrich (Oakville, ON, Canada)] for 30 min at 37 °C. Prior to the trimethylsilylation step, 40 µL of a retention time standard mixture was added that contained 3.7 % (w/v) of heptanoic acid, nonanoic acid, undecanoic acid, and tridecanoic acid; 7.4 % (w/v) of pentadecanoic acid, nonadeanoic acid and tricosanoic acid; 22.2 % (w/v) of heptacosanoic acid; and 55.5 % (w/v) of hentriacontanoic acid dissolved in tetrahydrofuran (10 g. L^{-1}).

3.2.5.2 GC-MS

Extracts were applied onto an Agilent 6890 gas chromatograph coupled to a HP model 5973 mass selective detector, split/splitless injector port and a HP 7683 series automatic sampler (Hewlett-Packard, Palo Alto, CA). Metabolite separation was carried out on a 30-m SPB-50 column with a 0.25-mm internal diameter and a 0.25-µm layer thickness (Superlco, Bellefonte, PA). The analysis was conducted under the following conditions: 1 µL of extract was injected in splitless mode, where the injector temperature was held at 250 °C; oven temperature was initially set at 70 °C for 5-min isothermal heating followed by a temperature ramp of 5 °C min^{-1} until a temperature of 310 °C was reached and ended with a final heating step for one additional min at 310 °C; helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. This program was then followed by a reconditioning step of 6 min

at 70 °C prior to the next injection. Full-scan spectra were recorded over an m/z 50-600 range at a rate of 2.7 scan. s^{-1}. Perfluorotributylamine (PFTPA), with m/z of 69, 219, and 502 was used for auto-tuning. The system was managed by the ChemStation software (Agilent Technologies, Santa Clara, CA) to validate the chromatograms and spectra.

3.2.5.3 Data Processing

GC-MS profiles were normalised using ribitol as the internal standard. Spectra were exported to AMDIS deconvolution and identification software (NIST, Gaithersburg, MD, USA), where peaks were deconvoluted and peak areas were calculated. The metabolites were identified after matching against customised reference spectrum databases including the National Institute of Standards and Technology (NIST 05) and the Golm metabolome database (GMD) (Kopka *et al.*, 2005) using a similarities ≥ 80 %. Some major constituents including sugars, organic and amino acids were further confirmed by comparing their mass spectra against the authentic reference compounds that were applied under the same conditions.

3.2.5.4 Data Analysis

The effects of growth temperature and harvest time, together with their interaction, were tested on amino acid concentrations, soluble sugar concentrations, soluble protein concentrations and proteolytic activities using two-way ANOVAs ($P < 0.05$). When ANOVAs revealed significant effects of the two factors or their interactions, Tukey HSD *a posteriori* tests were run to identify the treatments that differed. Variation across harvest times at each growth temperature regime was summarised by principal component analysis (PCA) separately for the phytohormones and metabolites and for the bulbs and the leaves, since they came from different samples and were expressed using different units (ng g^{-1} DM for phytohormones, relative units for the other metabolites). A biplot was produced for both phytohormones and metabolites, whereby a scores plot (representing each phytohormone or metabolite) was superimposed upon a loadings plot (representing each combination of growth temperature × harvest times) that was obtained from the PCA on the same graph. The biplots allowed us to graphically determine which phytohormones or metabolites were more strongly associated with each developmental stage (grouping of 2 or

more harvest times) or with growth temperature. PCA was performed on the sample variance-covariance matrix. Mixed model two-way ANOVAs were performed to assess the effect of growth temperature and harvest time on all identified phytohormones and other metabolites by using a false discovery rate (FDR)–adjusted criterion of $P = 0.01$ to take into account the number of analyses that were run.

Since most metabolites differed only as a function of harvest time, they were then clustered to identify groups of metabolites that behave similarly through time, regardless of growth temperature. Least significant differences (LSD) were used to represent the differences amongst the harvest times scaled by their variability. Partitioning was based on Non-Hierarchical Cluster Analysis (NHCA), using K-means clustering. The optimal number of clusters was identified based on the peak value of the Cubic Clustering Criterion (CCC). Compounds showing similar patterns were placed in the same profile graph. Each compound's profile was zeroed, based upon the mean of the first harvest time T1 and was also scaled by dividing the mean value by the compound's average SED (standard error of difference). Only clusters of metabolites that were associated with the last two stages according to PCA (S_L3 and S_L4 for the leaves; S_B3 and S_B4 for the bulbs) are included here. The other clusters are included as Supplementary material. Similarly, concentrations of phytohormones and relative abundances of the metabolites that were associated with the last two stages according to PCA, and which exhibited a significant effect of growth temperature or a significant interaction effect (growth temperature × harvest time) were plotted individually to illustrate their variation at the three growth temperatures as a function of harvest time. The individual metabolites that were associated with the earlier stages are included as Supplementary material. Statistical analyses and graphical data presentation were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and Prism 7.0 for Mac (Graphpad Software Inc., La Jolla, CA).

3.3 Results

3.3.1 Amino Acids, Soluble Proteins, Soluble Carbohydrates, and Proteolytic Activities

Leaf total amino acid concentrations slowly decreased throughout the season to about 30 % of the initial concentration by the end of the season (Fig. 3-1A). Similarly, bulb total amino acid concentration decreased by 75% over the growing season (Fig. 3-1B). For both leaves and bulbs, a more abrupt decrease was observed at the warmest growth temperature (Table 3-1). Leaf soluble protein concentrations were initially high, then decreased as leaves unfolded (T1 to T3), before remaining constant at least until leaves started to senesce (Fig. 3-1C). Similar variation was found in the bulbs at cooler temperatures, whereas for the plants grown at the warmest temperature, bulb soluble protein concentration was fairly stable throughout the season and always remained at much higher levels than at the two lower growth temperatures (Fig. 3-1D, Table 3-1).

Leaf soluble sugar concentrations slightly decreased during leaf unfolding, but increased gradually following complete leaf unfolding (T3) until the beginning of leaf senescence (T8) (Fig. 3-1E). Plants grown at the coolest temperature maintained the highest sugar levels once leaf unfolding was completed (Table 3-1). In contrast, bulb soluble sugar concentrations decreased gradually over time for all plants, and the difference amongst growth temperature regimes was generally not significant (Fig. 3-1F, Table 3-1).

Trypsin-like activity was apparent during leaf unfolding (Fig. 3-2D), whereas the other proteolytic activities were very low (Figs. 3-2A to 2C). A few days before the first visual sign of leaf senescence (around T5), cathepsin D/E-like activity started increasing at the higher temperature regime, and its activity strongly increased for all three growth temperatures once senescence became visible (at T8; Fig. 3-2B). At the onset of leaf senescence, proteolytic activities of all four enzymes were strongly stimulated.

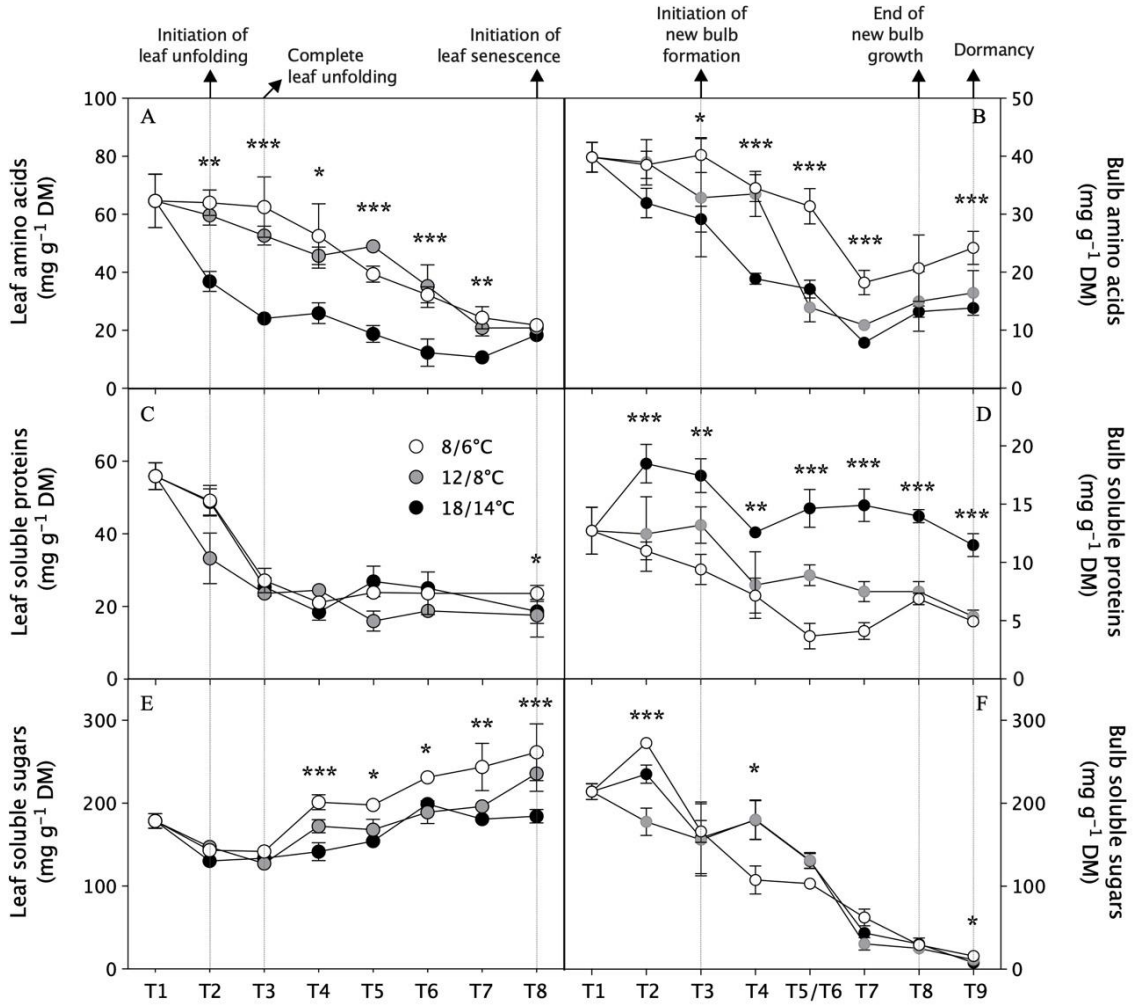


Figure 3-1. Total amino acid concentrations (mg g^{-1} DM, A and B), soluble protein concentrations (mg g^{-1} DM, C and D) and soluble sugar concentrations (mg g^{-1} DM, E and F) as a function of harvest time in leaves (A, C and E) and bulbs (B, D and F) of *E. americanum* plants grown under three growth temperature regimes. Means ± SEM (standard error of mean) of five plants are presented. *, ** and *** denote a significant difference among growth temperatures within each stage at $P < 0.05$, < 0.01 and < 0.001, respectively. Dotted lines indicate the following phenological stages, for leaves: initiation of leaf unfolding (T2), complete leaf unfolding (T3), and initiation of leaf senescence (T8); for bulbs: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).

Table 3-1. Results of two-way ANOVAs (*P* values) testing the effect of growth temperature and harvest time on amino acid concentrations, soluble sugar concentrations, soluble protein concentrations in the leaves and bulbs, and leaf proteolytic activities of *E. americanum* plants grown under three growth temperature regimes.

Variable	Growth temperature	Harvest time	Interaction (Temperature × Time)
Leaf			
Amino acids	**< 0.001**	**< 0.001**	**0.005**
Soluble sugars	**< 0.001**	**< 0.001**	0.051
Soluble proteins	**0.005**	**< 0.001**	0.230
Cathepsin B	0.168	**< 0.001**	0.367
Cathepsin D/E	0.309	**< 0.001**	**0.022**
Chymotrypsin	0.972	**< 0.001**	**0.042**
Trypsin	0.920	**< 0.001**	**0.004**
Bulb			
Amino acids	**< 0.001**	**< 0.001**	**< 0.001**
Soluble sugars	0.263	**< 0.001**	**0.009**
Soluble proteins	**< 0.001**	**< 0.001**	**0.030**

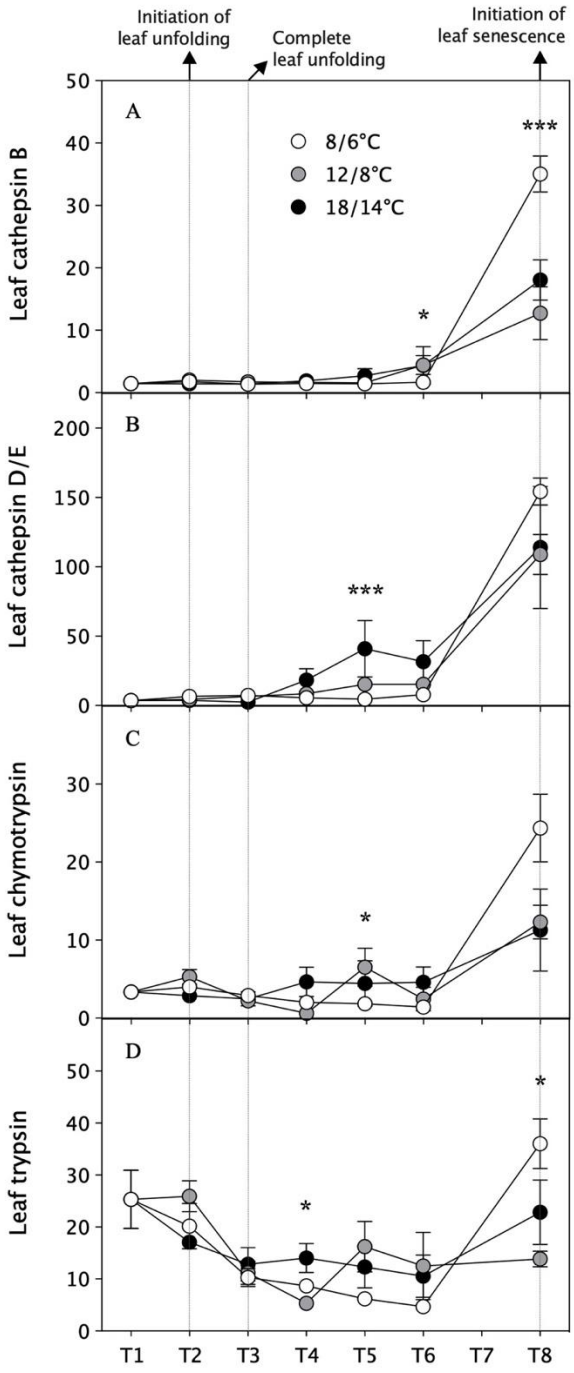


Figure 3-2. Proteolytic activities (relative fluorescence unit s^{-1} μg^{-1} protein) as a function of harvest time in leaves of *E. americanum* plants grown under three growth temperature regimes. Means ± SEM (standard errors of mean) of three plants are presented. * and *** denote a significant difference amongst growth temperatures within each stage at $P < 0.05$ and < 0.001, respectively. Dotted lines indicate the following phenological stages: initiation of leaf unfolding (T2), complete leaf unfolding (T3), and initiation of leaf senescence (T8).

3.3.2 Hormonal Profile

Seventeen phytohormones were identified in leaves and sixteen in the bulbs of *E. americanum* (H01 to H17; Tables 3-2 and 3-3). These included abscisic acid (ABA; H01), 1-aminocyclopropane-1-carboxylic acid, which is a precursor of ethylene (ACC; H02), 6 cytokinins (CKs) including two common active forms Z and 2-iP (H03 and H07) and four inactive forms (ZR, DHZ, DHZR, and iPA; H04, H05, H06 and H08), 5 or 6 gibberellins (GAs) [two active forms GA1 and GA4, and their precursors GA9, GA19 (present in the leaves only), GA20 and GA24; H09 to H14], indole-3-acetic acid (IAA; H15), jasmonic acid (JA; H16), and salicylic acid (SA; H17).

3.3.2.1 Leaf Profile

PCA was performed with leaf concentrations of the different phytohormones throughout the season from plants grown at the three growth temperatures. The analysis explained 38.5 % (Axis 1) and 24.2 % (Axis 2) of the overall variance (Fig. 3-3). PCA grouped harvest times (T1 to T8) into four main phenological stages along these two axes: (i) S_L1 was at the time of the transfer to the growth chamber (T1, common to all temperature regimes). (ii) S_L2 was the unfolding leaf period, which is divided into S_L2a (initiation of leaf unfolding, T2) and S_L2b (last stage of leaf unfolding: T3 for 8/6 ℃ and 12/8 ℃, T3 to T4 for 18/14 ℃). (iii) S_L3 was mature leaf period (T4 to T5 for 8/6 ℃, T4 to T6 for 12/8 ℃, and T5 to T7 for 18/14 ℃). (iv) S_L4 was the physiological onset of senescence (T6 to T8 for 8/6 ℃, T7 to T8 for 12/8 ℃, and T8 for 18/14 ℃).

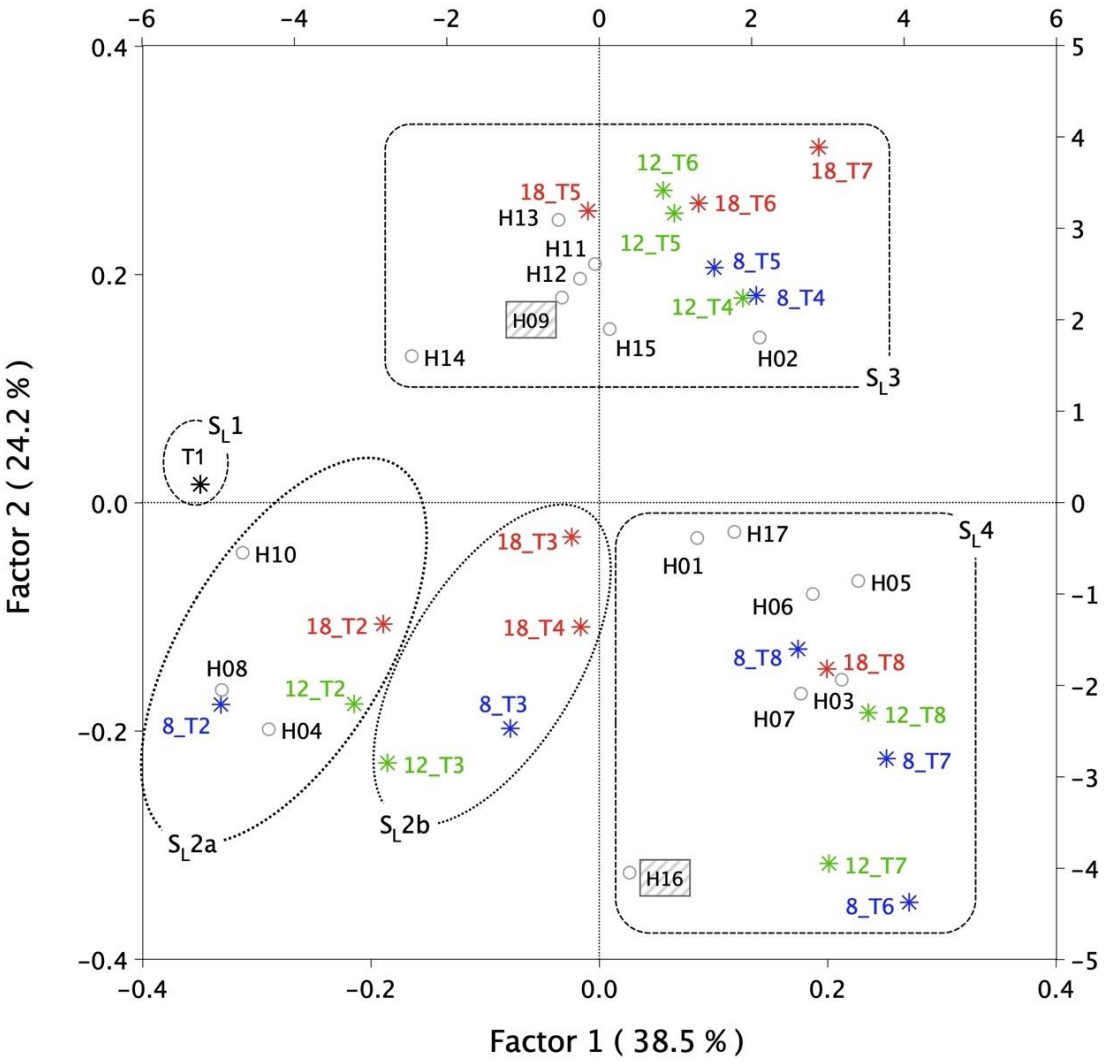


Figure 3-3. Biplot showing the first (horizontal) and second axis (vertical) of principal component analysis (PCA) for all phytohormones present in leaves of *E. americanum* (H01 to H17; see Table 3-2 for the list of phytohormones) and harvest times T1 to T8, for plants grown under three growth temperature regimes: 8/6 ℃ (8), 12/8 ℃ (12) and 18/14 ℃ (18). Harvest times were grouped into 4 main leaf phenological stages [S_L1, S_L2 (S_L2a and S_L2b)], S_L3 and S_L4), based upon their positions on the biplot. The variance explained by each component for each combination (Axis 1 and Axis 2) is given as a percentage in parentheses. Asterisks represent the positions of the different harvest times at 8/6 ℃ (blue), 12/8 ℃ (green) and 18/14 ℃ (red), and they are scaled against the lower left axis. T1 (black asterisk) is common to all temperature regimes. Circles represent the positions of the different hormones, and they are scaled against the upper-right axis. Boxes with slashes indicate the hormones that were not influenced by either growth temperature or harvest time (see Table 3-2 for statistical results).

The biplot (Fig. 3-3) indicated that ACC (H02), IAA (H15) and four GAs (H11 to H14) were associated with the mature leaf phase (S_L3), whereas ABA (H01), SA (H17) and four CKs [Z (H03), DHZ (H05), DHZR (H06), and 2-iP (H07)] were associated with the onset of the leaf senescence phase (S_L4) (Fig. 3-3). Most phytohormones varied in concentration over time and with growth temperatures (Table 3-2; significant temperature × time), and are thus presented individually as a function of harvest time in Figure 3-4. Four CKs, including the two active forms Z (H03) and 2-iP (H07), were low early in the season, but increased during the mature leaf stage and into the senescence stage (Figs. 3-4A and 4D). Four precursors of the active GAs (Figs. 3-4E to 4H) and IAA (H15; Fig. 3-4K) exhibited fluctuations through time, but they tended to remain high during the mature leaf phase until the onset of leaf senescence. The ethylene precursor ACC (H02) increased strongly at the two lower temperatures as leaves were maturing, then decreased again to low levels at the onset of leaf senescence (T7; Fig. 3-4J). Under the warmest temperature regime, the ACC concentration peak was delayed and occurred at the onset of leaf senescence. ABA (H01; Fig. 3-4I) and SA (H17; Fig. 3-4L) concentrations did not exhibit a great deal of variation during the leaf maturation period; both remained at low levels for most of the season, except for a clear sustained (ABA) or transient (SA) increase a few days before the onset of leaf senescence (T7) under the warmest temperature regime. SA steadily increased in the 12/8 ℃-grown plants from T5 to T8, but the overall change was much more modest than the peak observed in the 18/14 ℃-grown plants. Data for phytohormones that were associated with S_L2, namely ZR (H04), iPA (H08) and GA_4 (H10) are presented in supplementary figures (Annex 1).

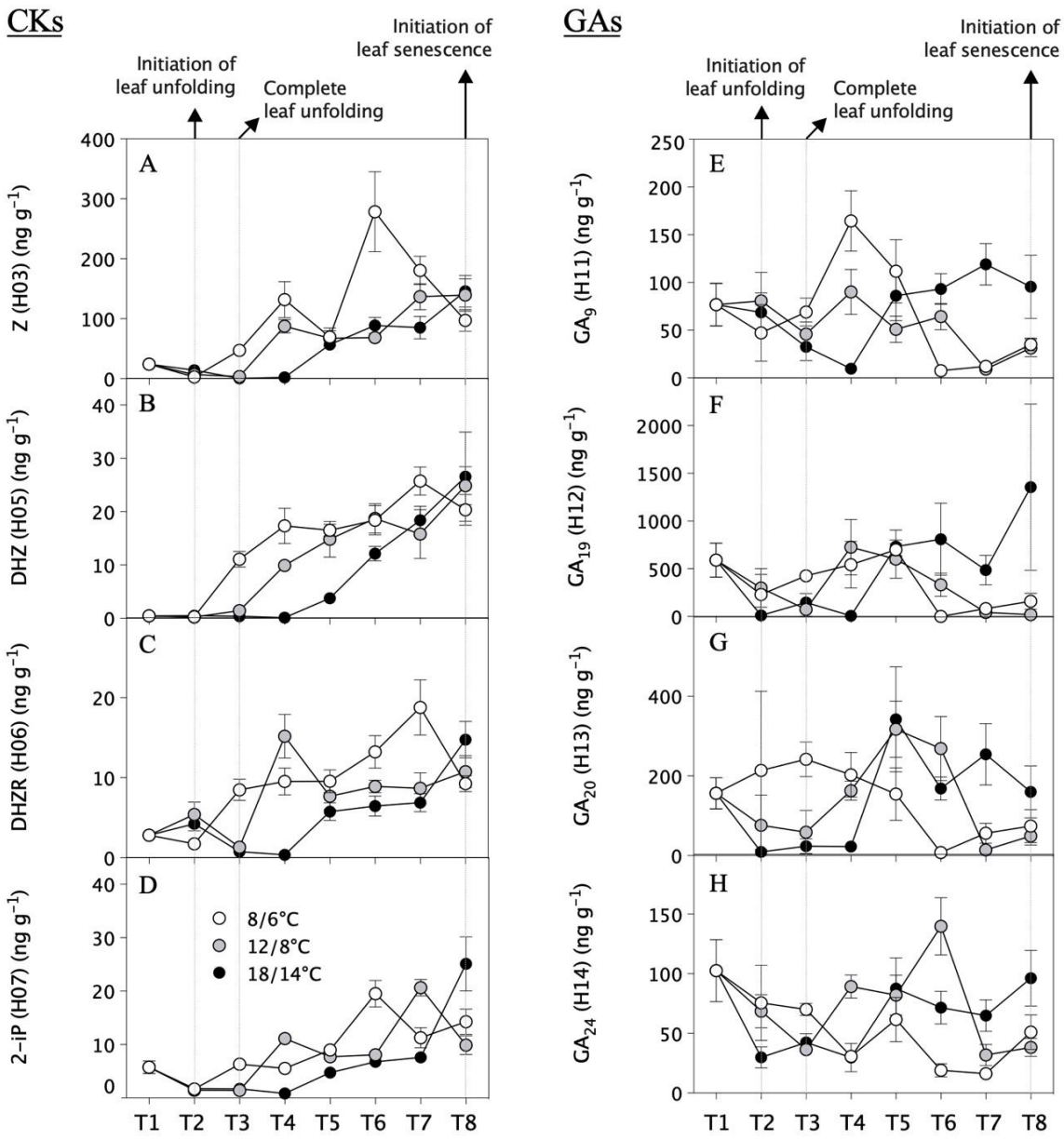


Figure 3-4. Changes in concentration (ng g^{-1}) of phytohormones over time within leaves of *E. americanum* plants grown under three growth temperature regimes. Only data from phytohormones that were associated with S_L3 and S_L4 [shown in bold in the column entitled "Phenological stage (PCA)" in Table 3-2], with a significant interaction effect (growth temperature × harvest time) are shown (see Table 3-2 for statistical results). Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of leaf unfolding (T2), complete leaf unfolding (T3), and initiation of leaf senescence (T8).

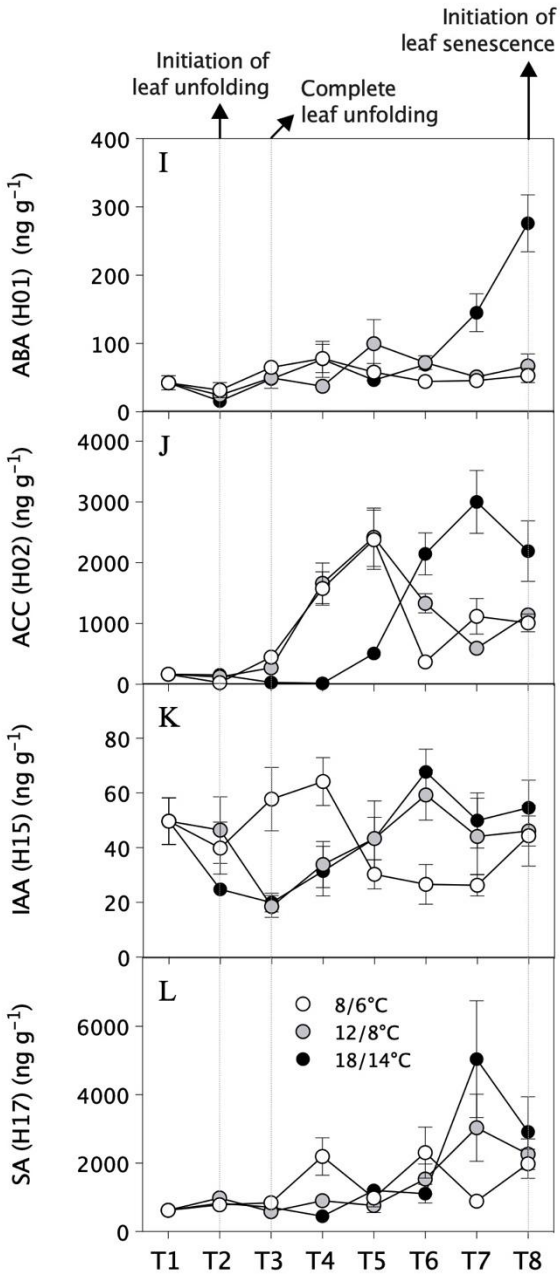


Figure 3-4. (continued)

3.3.2.2 Bulb Profile

The bulb PCA was performed with concentrations of the different phytohormones in the bulbs that were measured throughout the season from plants grown under the three temperature regimes. The first two principal components explained 44.9 % (Axis 1) and 8.9 % (Axis 2) of the overall variance (Fig. 3-5). Harvest times (T1 to T9) were grouped into four main phenological stages along these two axes. (i) S_B1 indicated plant transfer to the growth chamber (T1, common to all temperature regimes). (ii) S_B2 was the initiation of new bulb formation (T2 to T3). (iii) S_B3 was growth period of the new bulb (T4 to T7). (iv) S_B4 was termination of new bulb growth and entry into dormancy (T8 to T9). Changes in phytohormonal profiles took place at a similar harvest time for the three temperature regimes.

According to the biplot (Fig. 3-5), only one phytohormone, ABA (H01), was associated with the period of the new bulb growth (S_B3), while Z (H03) and GA_4 (H10) were associated with the end of its growth and its entry into dormancy (S_B4). Most phytohormones (13 of 16) varied in concentration over time, and with growth temperatures (Table 3-3; significant temperature × time). The other three phytohormones, viz., ABA (H01), DHZR (H06), and 2-iP (H07) varied through time only (Table 3-3; significant harvest time). ABA (H01) exhibited fluctuations through time, but tended to remain high during the period of the new bulb formation (T2 to T3, Fig. 3-6A). Both Z (H03) and GA_4 (H10) peaked at the initiation of the new bulb formation (T2, S_B2), and exhibited a clear sustained (Z) or transient (GA_4) increase at the end of the growth period of the new bulb and during its entry into dormancy (T9, S_B4) in the 18/14 ℃-grown plants (Figs. 3-6B and 6C). Data from phytohormones associated with S_B1 and S_B2 are presented in supplementary figures (Annex 2).

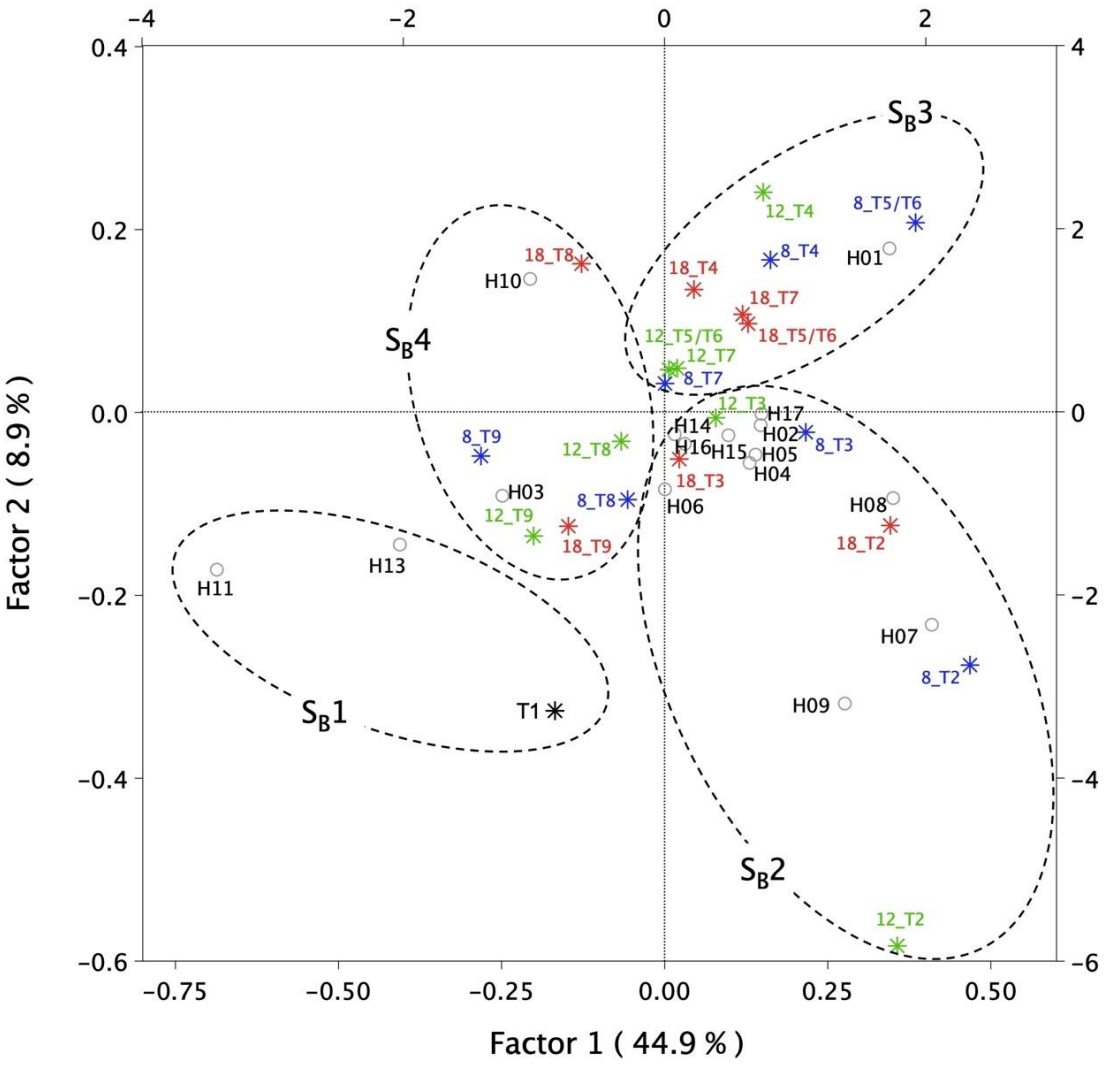


Figure 3-5. Biplot showing the first (horizontal) and second axis (vertical) of principal component analysis (PCA) for all phytohormones present in bulbs of *E. americanum* plants (H01 to H17; see Table 3-3 for the list of phytohormones) and harvest times T1 to T9, for plants grown under three growth temperature regimes: 8/6 ℃ (8), 12/8 ℃ (12) and 18/14 ℃ (18). Harvest times were grouped into 4 main bulb phenological stages (S_B1, S_B2, S_B3 and S_B4), based upon their positions on the biplot. The variance explained by each principal component for each combination (Axis 1 and Axis 2) is given as a percentage in parentheses. Asterisks represent the positions of the different harvest times at 8/6 ℃ (blue), 12/8 ℃ (green) and 18/14 ℃ (red), and they are scaled against the lower left axis. T1 (black asterisk) is common to all temperature regimes. Circles represent the positions of the different hormones, and they are scaled against the upper-right axis. Boxes with slashes indicate the hormones that were not statistically influenced by either growth temperature or harvest time (see Table 3-3 for statistical results).

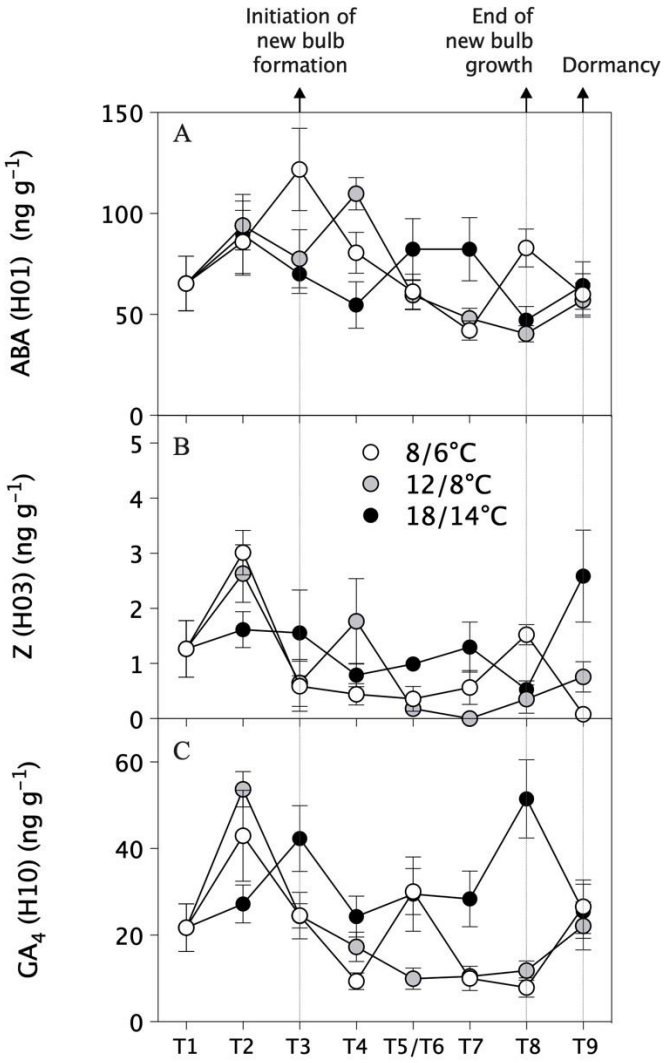


Figure 3-6. Changes in concentration (ng g^{-1}) of phytohormones over time within bulbs of *E. americanum* plants grown under three growth temperature regimes. Only data from phytohormones that were associated with S_B3 and S_B4 [shown in bold in the column entitled "Phenological stage (PCA)" in Table 3-3], with significant interaction effect (growth temperature × harvest time) are shown (see Table 3-3 for statistical results). Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).

3.3.3 Metabolomic Profile

Eighty metabolites were identified in the leaves of *E. americanum* (M01 to M80, Table 3-2), including 29 sugars and their derivatives (18 sugars and 11 sugar alcohols), 22 amino acids, 16 organic acids, and 5 fatty acids. A category that we referred to as "others" included 8 compounds of diverse biochemical origins. Eighty-nine metabolites were identified in the bulbs of *E. americanum* (M01 to M97; Table 3-3), including 29 sugars and their derivatives (20 sugars and 9 sugar alcohols), 22 amino acids, 24 organic acids, 5 fatty acids, and "others", which included nine compounds of different biochemical origins. Eight metabolites that were found in the leaves were absent from the bulbs, whereas seventeen of these (M81 to M97) were specific to bulb tissues. Leaf-specific metabolites included three sugar alcohols [arabitol (M08), maltitol (M52), and xylitol (M79)], two amino acids [norleucine (M60) and norvaline (M61)], two compounds with antioxidant activity, viz., 3-hydroxypyridine (M03) and threonic acid-1,4-lactone (M75), together with silanamine, N,N'-methanetetraylbis[1,1,1-trimethyl– (M70) (Table 3-2). Those compounds that were specific to the bulbs (M81 to M97) included eight organic acids, two amino acids, three sugars and sugar alcohols (glucopyranose, myo-inositol-2-phosphate, and rhamnose), together with N-acetyl glucosamine, uracil, and urea (Table 3-3).

3.3.3.1 Leaf Profile

A total of 80 leaf metabolites were included in PCA, the first two axes of which accounted for 33.7 % (Axis 1) and 14.1 % (Axis 2) of the overall variance, respectively (Fig. 3-7). This metabolite analysis exhibited groupings of the different harvest times that were similar those revealed by PCA for the leaf phytohormone data (Fig. 3-3). The same four developmental stages were identified: (i) S_L1 was plant transfer to the growth chambers (T1). (ii) S_L2 was the unfolding leaf period, which was divided into S_L2a (initiation of leaf unfolding, T2), and S_L2b (last stage of leaf unfolding, T3). (iii) S_L3 was the mature leaf period, which was subdivided into S_L3a (young mature leaves, T4 to T5 for 8/6 ℃, T4 for 12/8 ℃ and 18/14 ℃), and S_L3b (older mature leaves, T6 to T7 for 8/6 ℃, T5 to T6 for 12/8 ℃ and 18/14 ℃). (iv) S_L4 was physiological onset of leaf senescence (T8 for 8/6 ℃, T7 to T8 for 12/8 ℃ and 18/14 ℃).

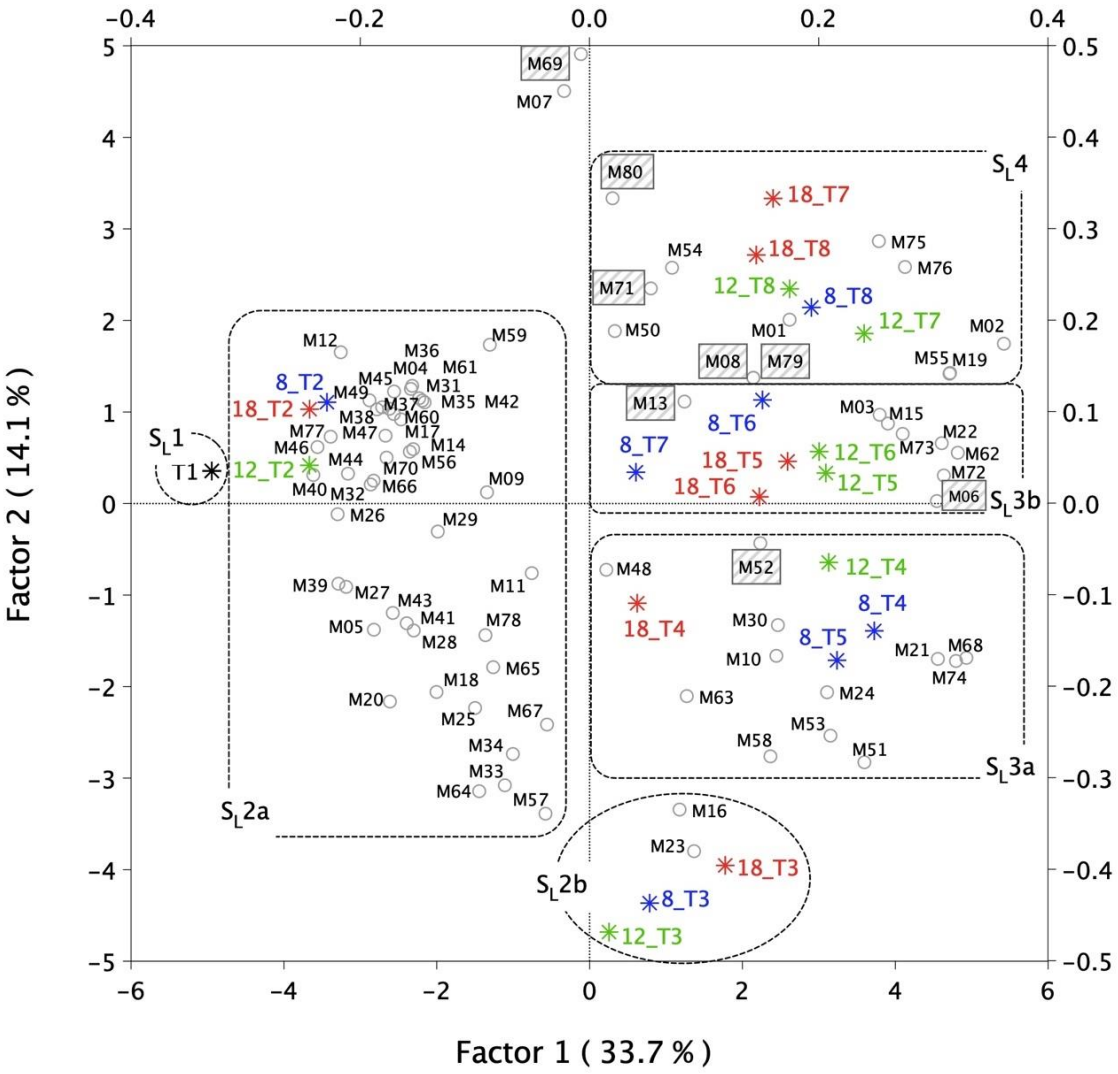


Figure 3-7. Biplot showing the first axis (horizontal) and second axis (vertical) of principal component analysis (PCA) for all identified metabolites present in leaves of *E. americanum* plants (M01 to M80; see Table 3-2 for the list of metabolites) and harvest times T1 to T8, for plants grown under three growth temperature regimes: 8/6 ℃ (8), 12/8 ℃ (12) and 18/14 ℃ (18). Harvest times were grouped into 4 main leaf phenological stages [S_L1, S_L2 (S_L2a and S_L2b), S_L3 (S_L3a and S_L3b) and S_L4], based on their positions on the biplot. The variance explained by each principal component for each combination (Axis 1 and Axis 2) is given as a percentage in parentheses. Asterisks represent the positions of the different harvest times at 8/6 ℃ (blue), 12/8 ℃ (green) and 18/14 ℃ (red), and they are scaled against the lower left axis. T1 (black asterisk) is common to all temperature regimes. Circles represent the positions of the different metabolites, and they are scaled against the upper-right axis. Boxes with slashes indicate the metabolites that were not statistically influenced by either growth temperature or harvest time (see Table 3-2 for statistical results).

Two-way ANOVAs on individual leaf metabolites indicated that most of them (57 out of 80) exhibited a simple effect of harvest time, and were subsequently grouped using a clustering method. There were 11 clusters in total. Figure 3-8 presented only those clusters grouping metabolites that were associated with the mature leaf stage (S_L3a and S_L3b), and the onset of leaf senescence (S_L4), as identified by the PCA (Fig. 3-7; also see Table 3-2). Three metabolites varied both with time and growth temperature, while 12 were influenced by the interaction between harvest time and growth temperature. Of these 15 metabolites, only those that were associated with the mature leaf phase (S_L3) and the onset of leaf senescence (S_L4) are presented individually as a function of harvest time in Figure 3-9. Due to their potential relationship with sink limitation, we also presented the two metabolites (fructose-6-phosphate, glucose-6-phosphate) that were associated with late leaf unfolding (S_L2b). Data for metabolites that were associated with S_L2a are presented as supplementary figures (Annex 3 and Annex 4). Eight metabolites were influenced neither by growth temperature nor by harvest time. These included seven sugars and sugar alcohols [arabitol (M08), erythritol (M13), maltitol (M52), ribose (M69), sorbitol (M71), xylitol (M79), and xylose (M80)], and one fatty acid [α-linolenic acid (M06)].

The two phosphorylated sugars, fructose-6-phosphate (M16) and glucose-6-phosphate (M23) were associated with the last stage of leaf unfolding (S_L2b), and exhibited a transient increase at T3, explaining their association with S_L2b in the PCA (Fig. 3-7: S_L2b, Fig. 3-8A, and Fig. 3-9C). The metabolites that were strongly associated with the young mature leaf phase (Fig. 3-7: S_L3a) were mainly sugars and sugar alcohols [gentiobiose (M21), isomaltose (M30), maltose (M53), myo-inositol (M58), raffinose (M68), and sucrose (M74)], and organic acids [glyceric acid (M24), lactic acid (M48), and malonic acid (M51)]. These metabolites are grouped into clusters 2 to 4 (Figs. 3-8B to 8D). Most exhibited higher abundances between T3 and T6 than earlier or later in the season. A saturated fatty acid, stearic acid (M63), and a phytosterol β-sitosterol (M10) were also linked to the young mature leaf phase (Fig. 3-7: S_L3a, Figs. 3-8D and 8E); their abundances were also higher during this developmental stage than either earlier or later in the season.

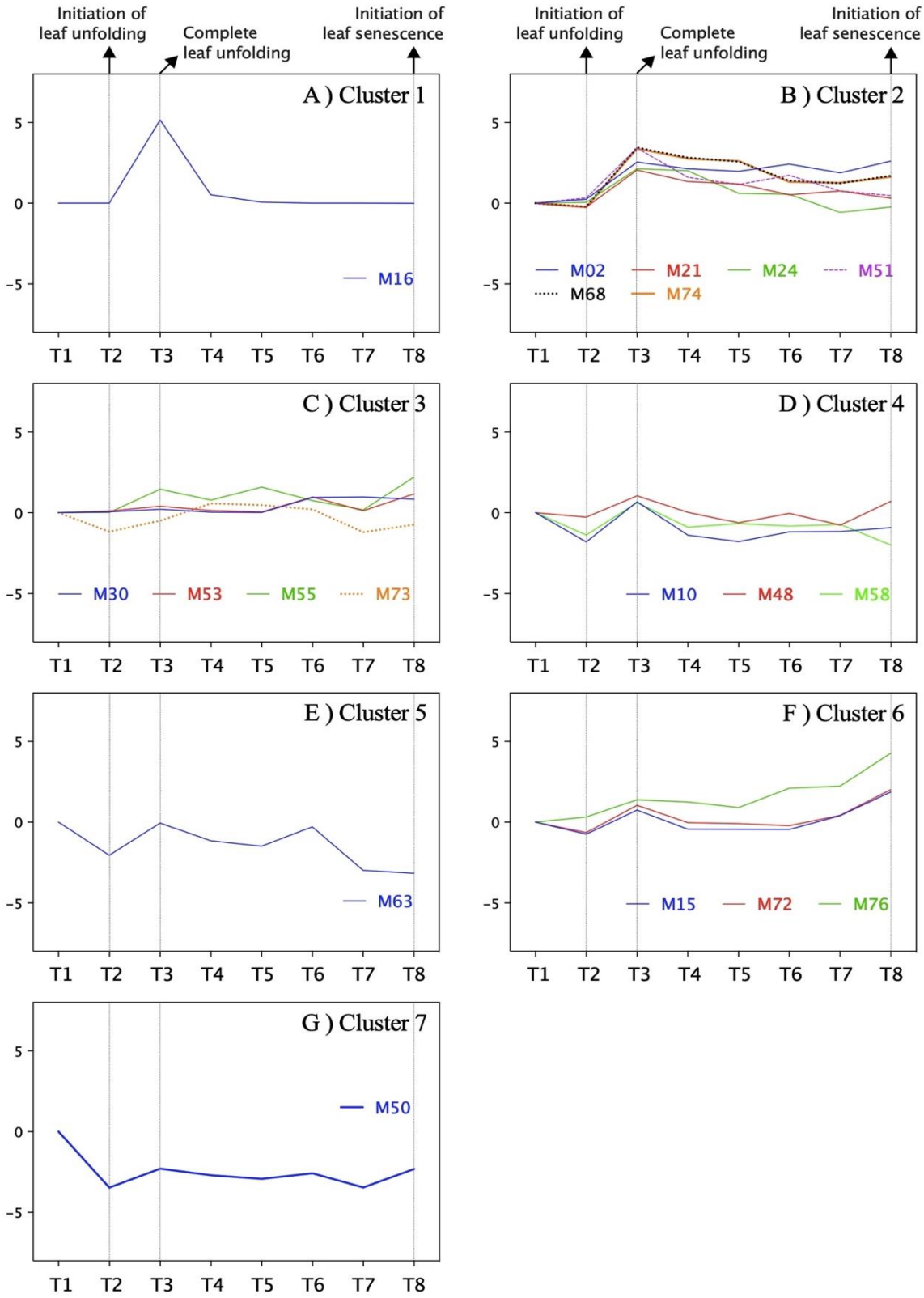


Figure 3-8. Clusters derived from hierarchical clustering analysis (HCA) showing relative variation in metabolite abundance in leaves of *E. americanum* plants as a function of

harvest time. The clusters were built using data from metabolites that exhibited a simple effect of harvest time (see Table 3-2 for statistical results). Each metabolite profile was zeroed on the mean of T1 and also scaled by dividing each value by the metabolite's average SED (standard error of difference). Only data from metabolites that were associated with S_L3 and S_L4 [shown in bold in the column entitled "Phenological stage (PCA)" in Table 3-2, plus M16] are shown. Dotted lines indicate the following phenological stages: initiation of leaf unfolding (T2), complete leaf unfolding (T3), and initiation of leaf senescence (T8).

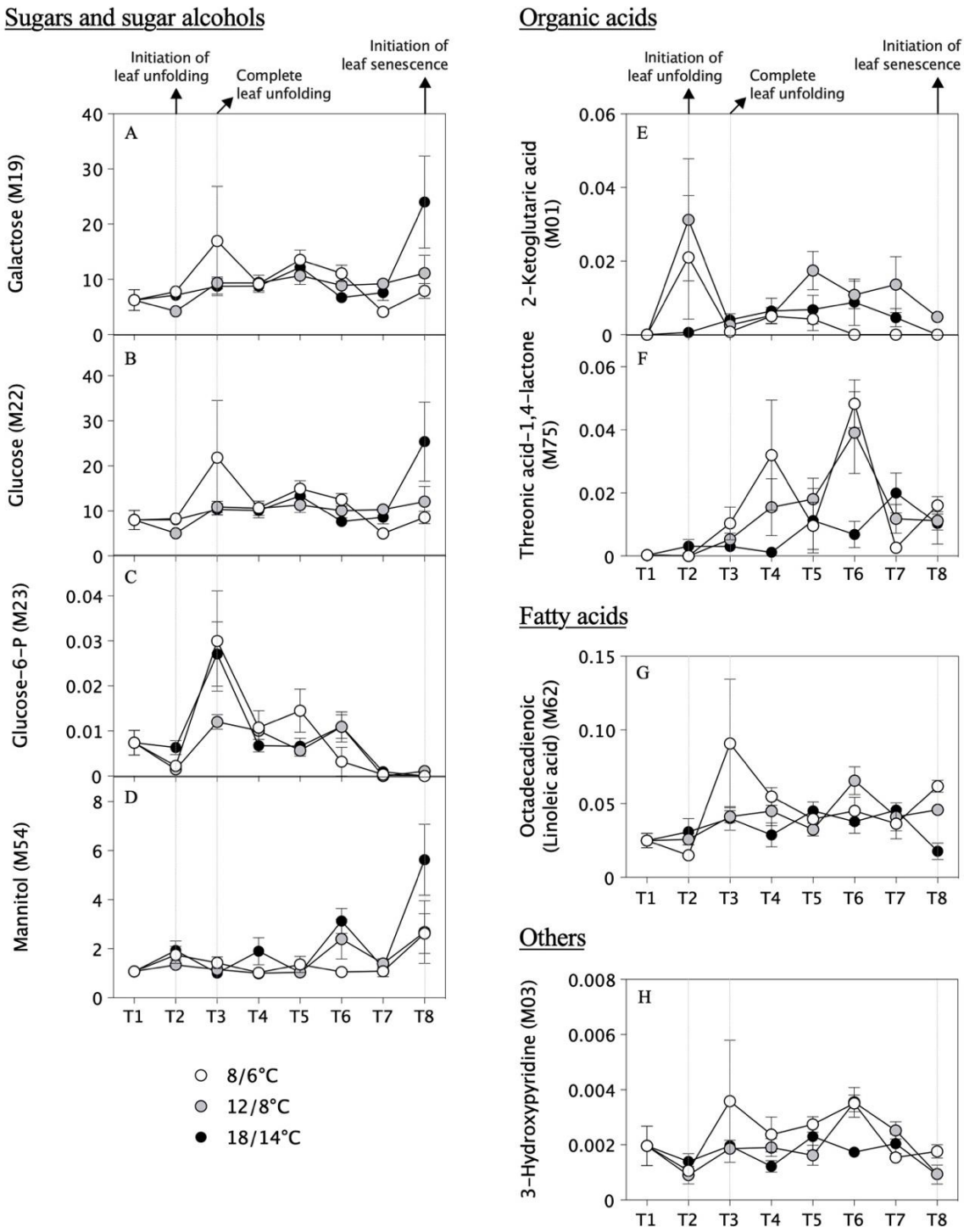


Figure 3-9. Relative abundance of metabolites in leaves of *E. americanum* plants grown under three growth temperature regimes as a function of harvest time. Only data from metabolites that were associated with S_L3 and S_L4 [shown in bold in the column entitled "Phenological stage (PCA)" in Table 3-2, plus M23], with a significant interaction effect

(growth temperature × harvest time), are shown (see Table 3-2 for statistical results). Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of leaf unfolding (T2), complete leaf unfolding (T3), and initiation of leaf senescence (T8).

Few metabolites were associated with the older mature leaf phase (Fig.3-7: S_L3b). They included three sugars [fructose (M15), glucose (M22), and sorbose (M72)], an organic acid [succinic acid (M73)], a fatty acid [linoleic acid (M62)], and a pyridine derivative [3-hydroxypyridine (M03)]. Fructose (M15) and sorbose (M72) clearly increased from T6 until the end of the season (Fig. 3-8F), whereas glucose (M22) increased in abundance a few days later (from T7) (Fig. 3-9B). The organic acid (M73) decreased towards the end of the season (Fig. 3-8C), whereas 3-hydroxypyridine (M03) and linoleic acid (M62) were higher during the older mature leaf phase than earlier or later on (Figs. 3-9G and 9H).

Eight metabolites were associated with the physiological onset of leaf senescence (Fig.3-7: S_L4). All exhibited an increase prior to leaf senescence onset (M02, M50, M55, and M76, Figs. 3-8B, 8C, 8F, and 8G; M19, M54, and M75, Figs. 3-9A, 9D, and 9F), except for the organic acid 2-ketoglutaric acid (M01, Fig. 3-9E). M01 decreased in abundance towards the end of the season. Those compounds that increased in abundance were the sugars or sugar alcohols [2-O-glycerol-β-D-galactopyranoside (M02), galactose (M19), mannitol (M54), and mannose (M55), and organic acids [malic acid (M50), and threonic acid-1,4-lactone (M75), which is a product of ascorbic acid catabolism], together with an important antioxidant, tocopherol (M76).

3.3.3.2 Bulb Profile

PCA of the bulbs was performed on 89 metabolites. The first two principal components respectively accounted for 23.7 % (Axis 1) and 16.8 % (Axis 2) of the total variance (Fig. 3-10). This analysis resulting in groupings of the different harvest times that were similar to the PCA on bulb phytohormones (Fig. 3-5). We thus used the same four developmental stages to group them: (i) S_B1 was plant transfer to the growth chamber (T1, common to all temperature regimes). (ii) S_B2 was initiation of the new bulb formation (T2 to T3). (iii) S_B3 was new bulb growth (T4 to T7). (iv) S_B4 was the end of new bulb growth and entry into dormancy (T8 to T9). Changes from one developmental stage to the next took place at the same harvest time for all three temperature regimes, as also reported with the bulb phytohormone data.

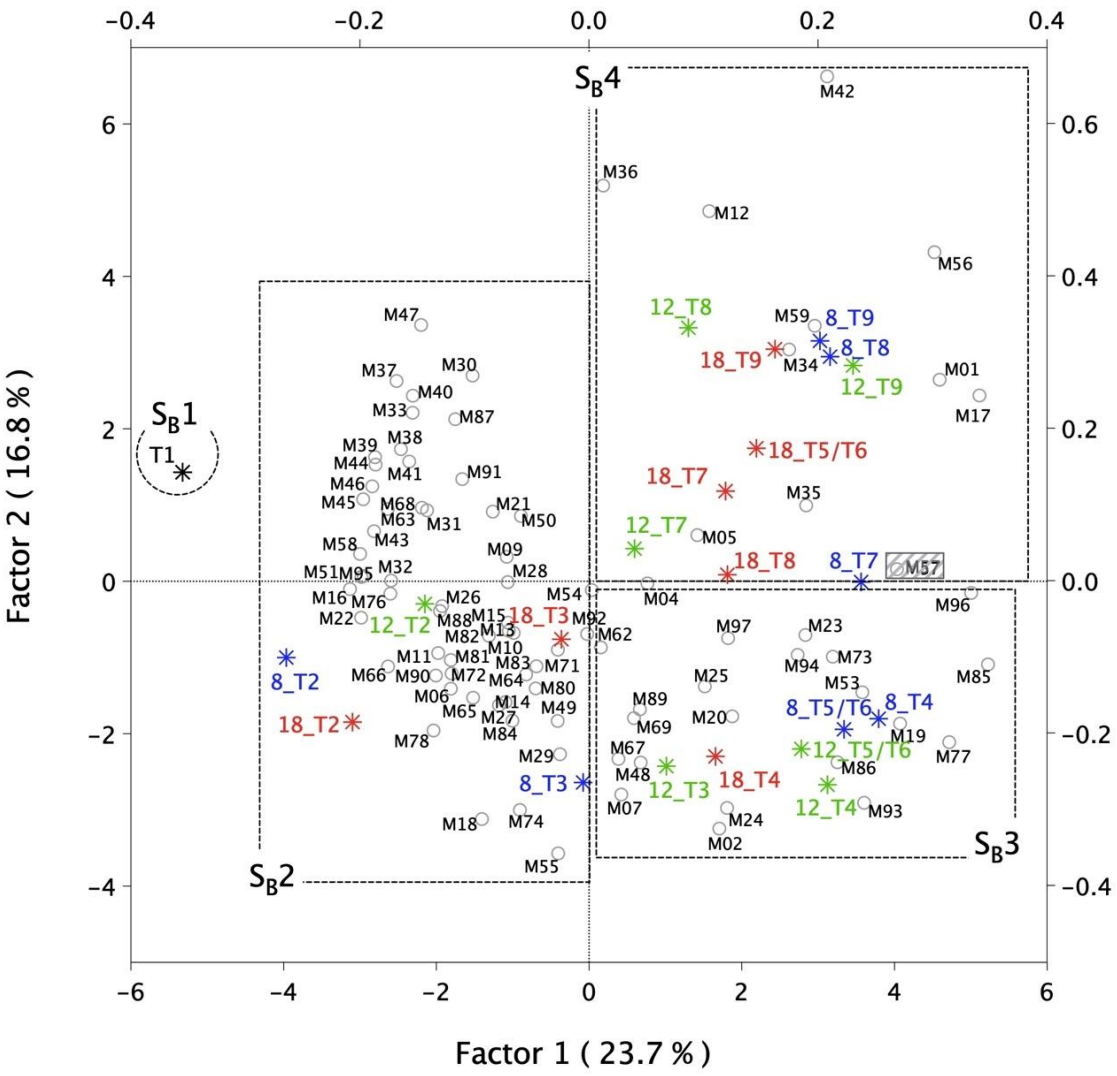


Figure 3-10. Biplot showing the first axis (horizontal) and second axis (vertical) of principal component analysis (PCA) for all metabolites present in bulbs of *E. americanum* plants (M01 to M97; see Table 3-3 for the list of metabolites) and harvest times T1 to T9, for plants grown under three growth temperature regimes: 8/6 ℃ (8), 12/8 ℃ (12) and 18/14 ℃ (18). Harvest times were grouped into 4 main bulb phenological stages (S_B1, S_B2, S_B3 and S_B4), based upon their positions on the biplot. The variance explained by each principal component for each combination (Axis 1 and Axis 2) is given as a percentage in parentheses. Asterisks represent the position of the different harvest times at 8/6 ℃ (blue), 12/8 ℃ (green) and 18/14 ℃ (red), and they are scaled against the lower left axis. T1 (black asterisk) is common to all temperature regimes. Circles represent the positions of the different metabolites, and they are scaled against the upper-right axis. Boxes with slashes indicate the metabolites that were not statistically influenced by either growth temperature or harvest time (see Table 3-3 for statistical results).

Two-way ANOVAs on individual metabolites indicated that nearly one-third (24 of 89) exhibited a simple effect of harvest time, after which they were grouped using a clustering method. Of the 11 clusters that were formed, only grouping of metabolites that were associated with the new bulb growth period (S_B3) and the end of new bulb growth and its entry into dormancy (S_B4) are presented in Figure 3-11, and which were identified by the PCA (Fig. 3-10). Most metabolites (58 of 89) were affected by the interaction between harvest time and growth temperature. Six other metabolites varied both with time and growth temperature. Only those associated with the last two growth phases, i.e., S_B3 and S_B4, are presented individually as a function of harvest time (Fig. 3-12). Like the leaf metabolomics data, given their potential relationship to sink limitation, phosphorylated sugar fructose-6-phosphate (M16) was presented in Fig. 3-12A, as it also was associated with initiation of new bulb formation (S_B2). Data for all the metabolites that were associated with S_B2 are presented as supplementary figures (Annexes 6 to 9). Only one metabolite, melibiose (M57), was influenced by neither harvest time nor growth temperature and, therefore, its data are not shown graphically.

Fructose-6-phosphate (M16) exhibited a transient increase at the initiation of new bulb formation (Fig. 3-10: S_B2) in plants grown under higher temperatures (Fig. 3-12A). About one-quarter of the metabolites (22 of 89) were strongly associated with the period of new bulb growth (Fig. 3-10: S_B3); these included nine sugars and sugar alcohols [2-O-glycerol-β-D-galactopyranoside (M02), arabinose (M07), galactose (M19), galactosylglycerol (M20), glucose-6-phosphate (M23), glycerol (M25), maltose (M53), ribose (M69), and trehalose (M77)], eight organic acids [glyceric acid (M24), lactic acid (M48), pyruvic acid (M67), succinic acid (M73), butyric acid (M85), gluconic acid (M86), methylene succinic acid (M89), and nonanoic acid (M93)], two unsaturated fatty acids [linoleic acid (M62) and oleic acid (M94)], an amino acid [GABA (M04)], and two metabolites from other classes [uracil (M96) and urea (M97)]. Temporal changes in these metabolites are included in clusters 1 to 4 (Figs. 3-11A to D), and in Fig.3-12 (Figs. 3-12B to 12E, 12G, 12H, 12K, 12O, and 12Q to 12T). Most exhibited a higher abundance between T4 and T7 than earlier or later in the season, except for urea (M97). There was a transient increase in urea at the beginning of new bulb growth (at T3, Fig. 3-11A).

Ten metabolites were associated with growth termination of the new bulb and its entry into dormancy (Fig. 3-10: S_B4). Half exhibited an increase in abundance from T8 to T9 [L-glutamic acid (M34), Fig.3-11D; 2-ketoglutaric acid (M01), fumaric acid (M17), melezitose (M56), and nicotinic acid (M59); Figs.3-12F, I, J, L to N, P, and U]. Three metabolites exhibited the same increase, but only in plants grown under 18/14 ℃ regime (Figs. 3-12J, 12M and 12U). Two amino acids exhibited either a transient increase at the beginning of leaf senescence [L-proline (M42); Fig. 3-11E] or a decrease in abundance from T8 to T9 [L-glutamine (M35); Fig.3-12I].

In many instances, metabolites that belonged to a particular category tended to behave similarly within the leaves or the bulbs. Amino acids abundances were high early in the season, then decreased thereafter in both leaves (Annexes 3A to 3C: clusters 1 to 3, Annexes 4A to 4E) and bulbs (Annex 6). In leaves, most sugars and sugar alcohols increased over time (Figs. 3-8B to 8F: clusters 2 to 6), and, thus, were associated with the mature leaf period and the onset of leaf senescence. In bulbs, most sugars and sugar alcohols decreased over time (Fig. 3-12 and Annex 8), and were associated with the new bulb growth period, the end of new bulb growth, and its entry into dormancy. In leaves, most organic acids were more abundant early than later in the season (Annexes 3D and 3E: clusters 4 and 5), whereas their abundances in the bulbs were associated with new bulb growth (Figs.3-12 M to 12T, Annex 7).

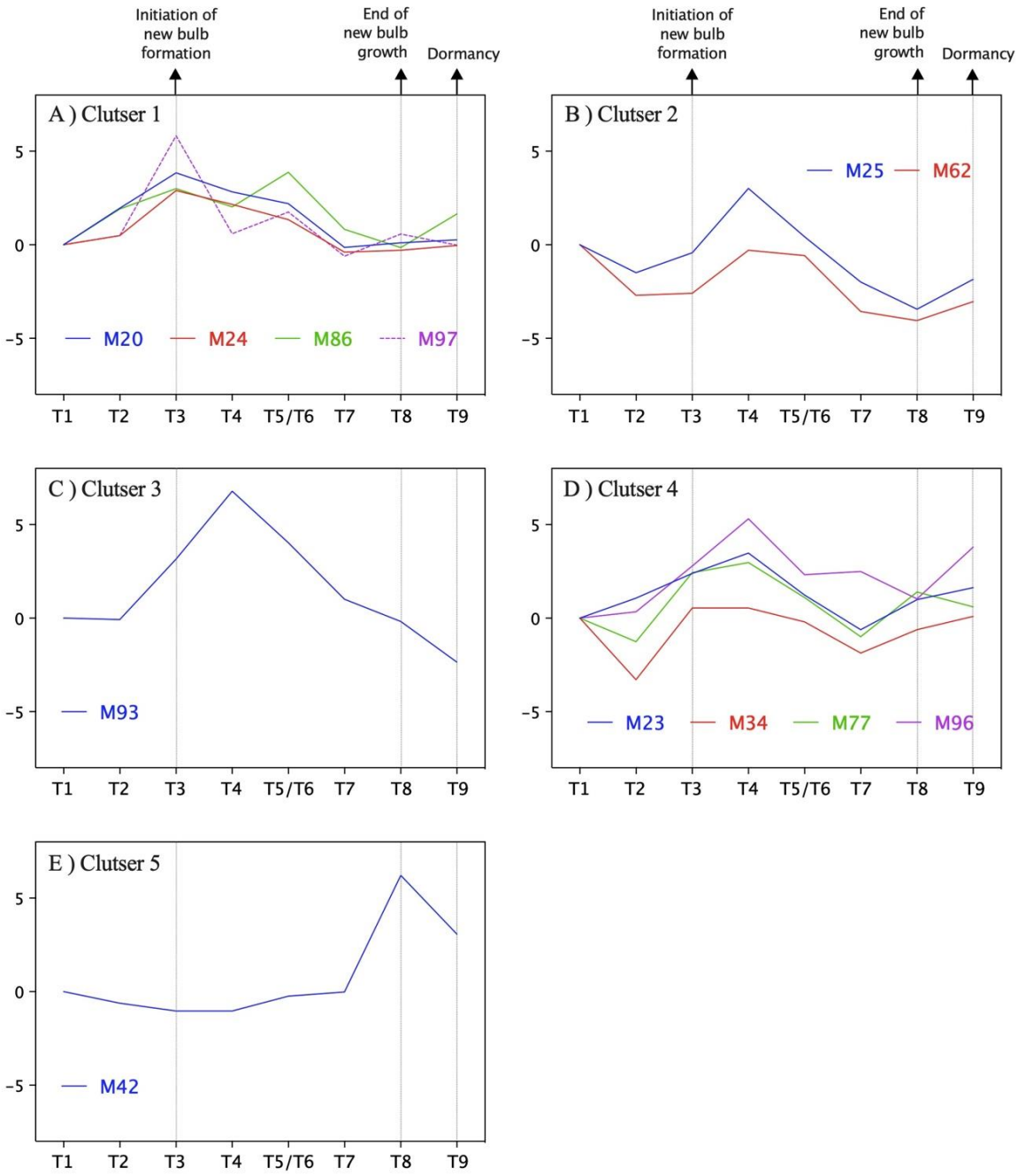


Figure 3-11. Clusters derived from hierarchical clustering analysis (HCA) showing relative variation in metabolite abundance in bulbs of *E. americanum* plants as a function of harvest time. The clusters were built using data from metabolites that exhibited a simple effect of harvest time (see Table 3-3 for statistical results). Each metabolite profile was zeroed on the mean of T1 and also scaled by dividing each value by the metabolite's average SED (standard error of difference). Only data from metabolites that were associated with S_B3 and S_B4 [shown in bold in the column entitled "Phenological stage (PCA)" in Table 3-3]

are shown. Dotted lines indicate the following phenological stages: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).

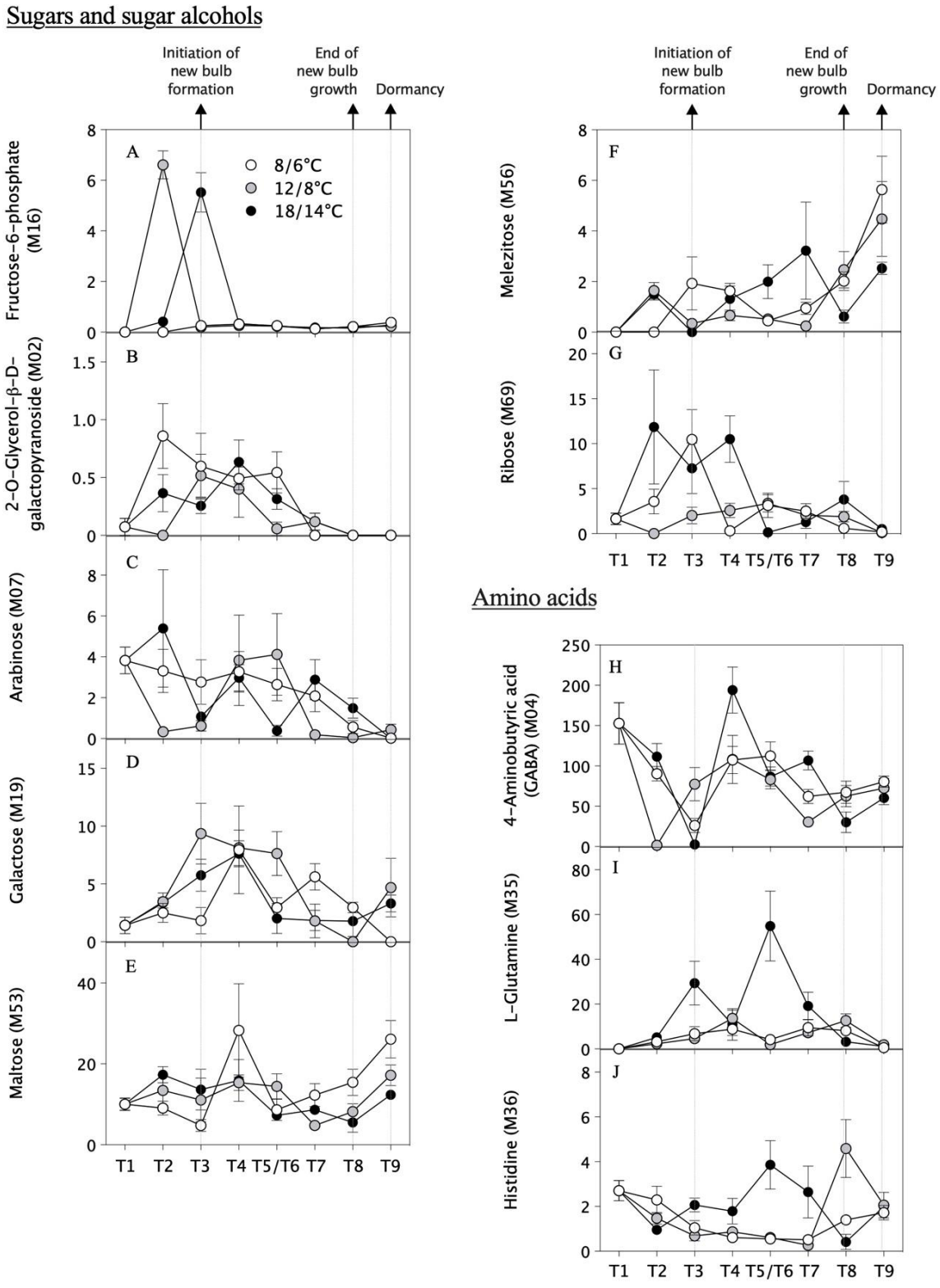


Figure 3-12. Relative abundance of metabolites in bulbs of *E. americanum* plants grown under three growth temperature regimes as a function of harvest time. Only data from

metabolites that were associated with S_B3 and S_B4 [shown in bold in the column entitled "Phenological stage (PCA)" in Table 3-3, plus M16], with significant interaction effect (growth temperature × harvest time) are shown (see Table 3-3 for statistical results). Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).

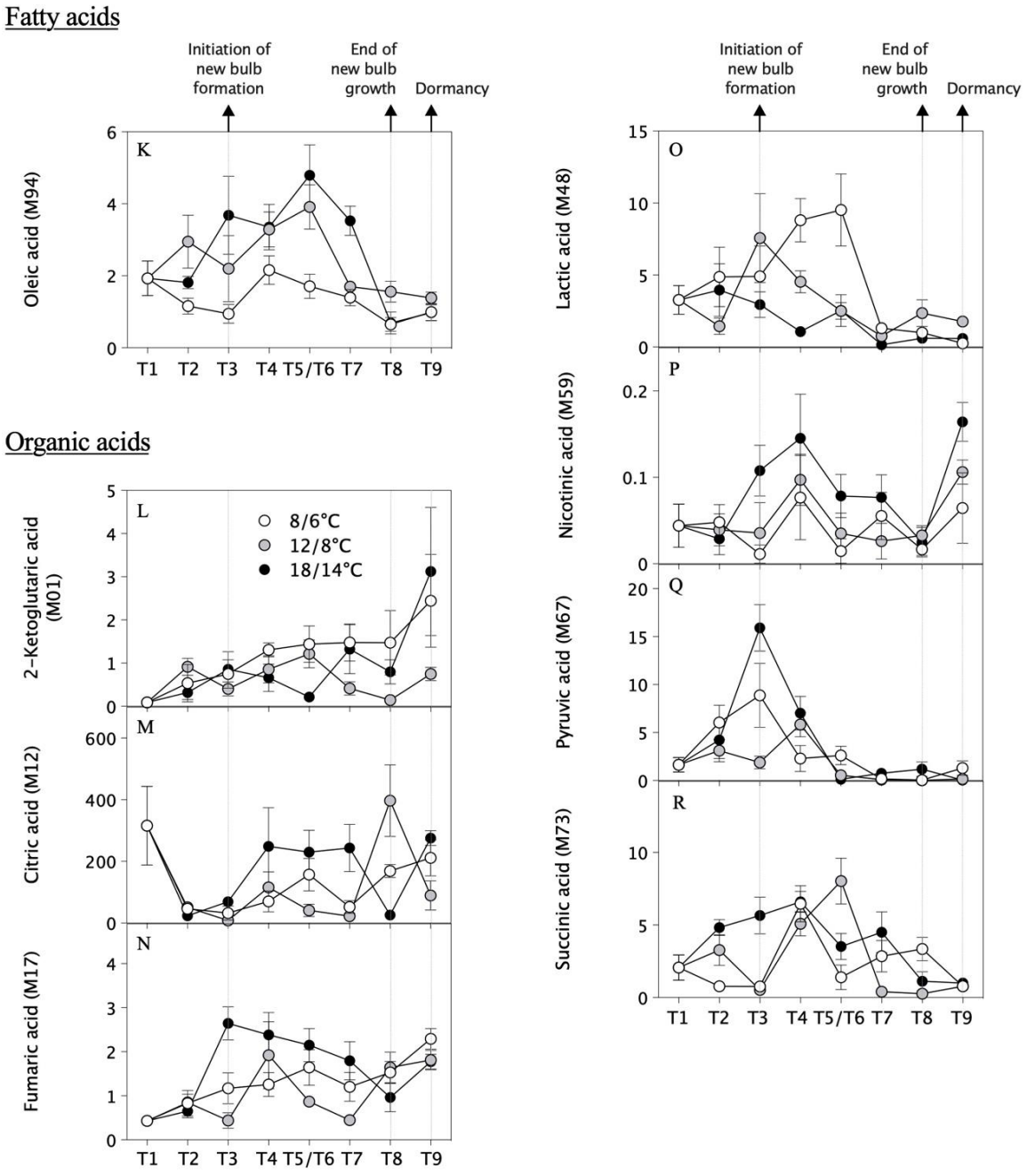


Figure 3-12. (continued)

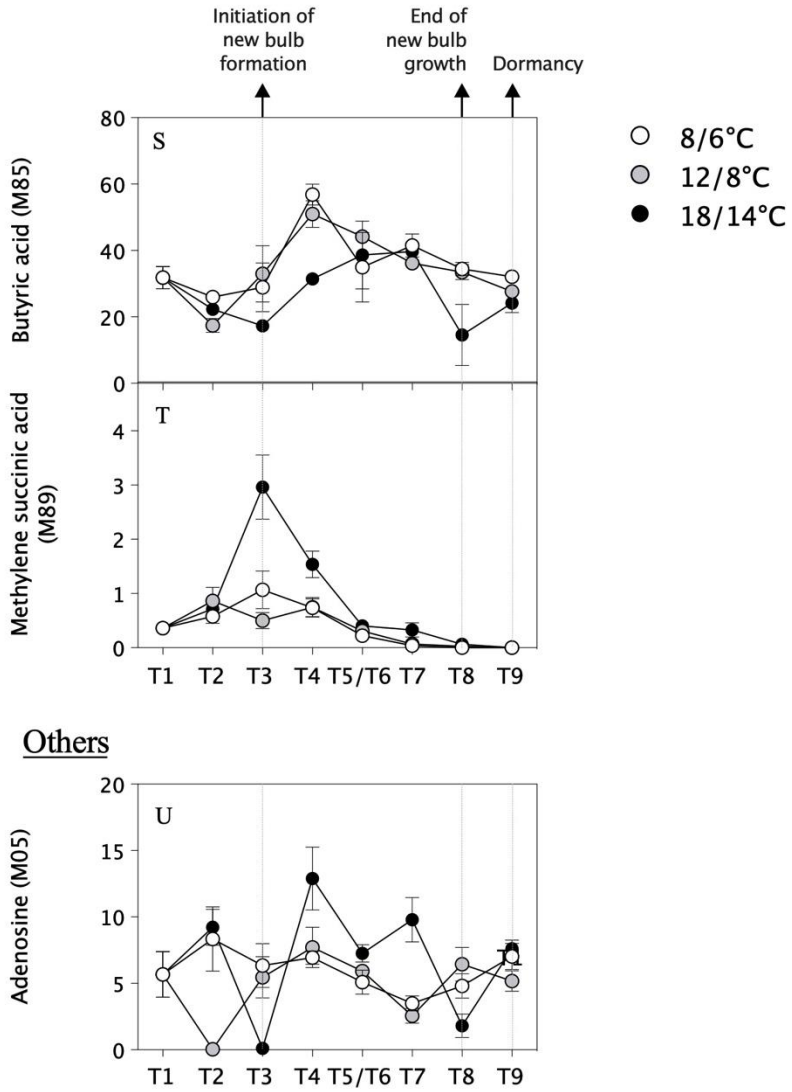


Figure 3-12. (continued)

3.4 Discussion

The phytohormonal and metabolomic profiles of young expanding leaves, mature leaves, and leaves undergoing senescence were very distinct from one another, supporting the pre-defined growth stages that had been used to establish the harvest schedule based upon visual assessments. High proteolytic activities that we detected at the onset of leaf senescence confirmed that the first visual sign of chlorophyll degradation corresponds to the very beginning of nitrogen remobilisation, since we did not yet record a significant decrease in soluble protein content. Profiles at the bulb level were also tightly associated with specific developmental stages: the old bulb translocated carbon and nitrogen reserves to the shoot; the initiation of the new bulb; growth; and carbohydrate accumulation in the new bulb and its entry into dormancy. Some metabolites are expected to behave similarly at leaf and bulb levels, if indeed there are signalling molecules that synchronise source and sink activity.

However, many metabolites and phytohormones will expected to behave differently in these two organs within a single developmental stage, given that the main metabolic pathways in the source and sink are different (Ho, 1988; Foyer and Paul, 2001). Developmental stages also differ between the two organs (leaf senescence *vs.* bulb dormancy, mature leaves *vs.* growing bulbs). Indeed, most phytohormones in the leaves were associated with the later stages of leaf growth (S_L3 and S_L4), whereas those in the bulbs were associated with its earlier growth stages (S_B2). Abundance of amino acids generally decreased over time in both leaves and bulbs, while most organic acids were more abundant during the most active growth periods, which were respectively leaf unfolding and new bulb growth. The most likely candidates for the source–sink signalling pathway at the leaf level are sugars and sugar alcohols, given that most increased in abundance towards the end of the season leading to leaf senescence.

3.4.1 Changes at the Leaf Level

Zeatin was the dominant CKs in mature *E. americanum* leaves, as has been frequently reported in higher plants (Lim *et al.*, 2007). CKs are known to delay leaf senescence

(Roitsch and Ehneß, 2000). Further, application of GAs has been shown to delay both leaf and flower senescence in lilies (Ranwala and Miller, 1998; Arrom and Munné-Bosch, 2012). Increased levels of IAA have been associated with delays in senescence, although contradictory results also have been reported in the literature (Mueller-Roeber and Balazadeh, 2014). Both GAs and IAA levels varied during the mature leaf phase, but they generally remained high towards the onset of leaf senescence, especially under the highest temperature regime. High levels of CKs, GAs, and IAA in mature leaves might thus counteract the increasing abundances of soluble sugars at least for a while, thereby avoiding induction of early leaf senescence.

At the highest growth temperature, we reported a strong increase in ABA, ACC, and SA a few days before leaf senescence. These hormones could likely trigger leaf senescence according to the "senescence window" concept which argues that age-related changes are considered to be primary regulator of leaf senescence (Jing *et al.*, 2002; Schippers *et al.*, 2007; Schippers *et al.*, 2015). During leaf aging, developmental cues lead to the diminished action of the senescence-retarding hormones such as auxin, GAs, and CKs, together with concomitant strengthening of action of senescence enhancing hormones, such as ethylene, ABA and SA. The peak in ACC a few days ahead of the first sign of leaf senescence suggests that ethylene might be the principal phytohormone triggering leaf senescence in *E. americanum*, but only at the highest temperature, where leaf senescence occurs earlier than at the two lower temperatures. A signalling role for ACC that is independent of ethylene has been suggested in recent findings in *Arabidopsis* mutant analysis (Yoon and Kieber, 2013; Van de Poel and Van Der Straeten, 2014). Its potential role in inducing leaf senescence, independent of ethylene, needs further investigation.

Another possible explanation for the difference in phytohormonal profiles amongst growth temperatures is the rate at which metabolic changes take place. Plants were harvested more frequently at higher than at lower temperatures (every 2, 3 or 4 days at 18/14 °C, 12/8 °C, and 8/6 °C, respectively) to complete the same number of harvests for different leaf life durations. Therefore, similar metabolic changes between harvests means in fact faster changes at higher than at lower temperatures. Faster metabolic changes may

more easily trigger phytohormonal cascades than would much slower metabolomics changes that took place under the lowest growth temperature. Finally, hormones that were not analysed in the present study, such as the brassinosteroids and strigolactones, have been shown to be involved in the process of leaf senescence (Jeong *et al.*, 2010; Khan *et al.*, 2014; Yamada and Umehara, 2015). Their actions and possible roles at lower temperature need further investigations.

As has been reported previously in *Arabidopsis*, the most distinctive metabolomic profile was found in developing leaves (48 out of 80 metabolites that were associated with S_L2 phase), whereas the profile of mature leaves slowly shifts as leaves ages and enter senescence (Watanabe *et al.*, 2013). High soluble protein and amino acid levels were detected in young leaves; their decrease with age is consistent with the results that were reported in *Arabidopsis* (Diaz *et al.*, 2008) and trembling aspen (*Populus tremuloides* Michx.) (Jeong *et al.*, 2004). Minor amino acids, such as GABA (M04), L-isoleucine (M37), L-leucine (M38), and L-tyrosine (M46), have been shown to accumulate dramatically with leaf aging in *Arabidopsis* (Diaz *et al.*, 2005). This response might be related to amino acid recycling, and nitrogen mobilisation. Diaz *et al.* (2005) also suggested that GABA could be involved in nitrogen storage and in anaplerotic reactions. Yet, in the present study, these metabolites decreased with age like other amino acids, consistent with the soluble protein data, which suggested that nitrogen mobilisation did not start prior to the first visual sign of chlorophyll degradation.

We reported a transient increase in the abundance of two phosphorylated sugars, fructose-6-phosphate (M16) and glucose-6-phosphate (M23), not only at the end of leaf unfolding (S_L2b; Figs. 3-8A and 3-9C, respectively), but also in the bulbs around the same time (S_B2 and S_B3; Figs. 3-12A and 3-11D, respectively), along with myo-inositol-2-phosphate (M90, Annex 8K). Fructose- and glucose-6-phosphate were negatively correlated (-0.177 and -0.175, respectively) with biomass accumulation in shoots of *Arabidopsis thaliana* (L.) Heyhn (Meyer *et al.*, 2007). Their transient accumulation in the present study suggested temporary sink limitation during new bulb construction. At T3, the leaves already exhibited relatively high photosynthetic rates (Gandin *et al.*, 2011), whereas

the new bulb was still very small when present. We posited that the mother bulb (old bulb), which still contains some carbohydrates, could partially supply the needs of the daughter bulb (new bulb), explaining the transient accumulation of glucose-6-phosphate and fructose-6-phosphate in the leaves. As soon as the new bulb increased in size and started to accumulate carbohydrates, levels of these sugars would strongly decrease in the leaves and, to a lesser extent, in the bulbs at least for glucose-6-phosphate.

Sucrose (M74) is known as the major transport sugar, although many species translocate the raffinose (M68) series (i.e., galactose-glucose-fructose trisaccharides), along with sucrose (Zimmermann, 1957; Turgeon, 1989). High levels of sucrose and raffinose that were observed during the mature leaf stage were expected, since the latter corresponds to the period of high photosynthetic rates (Gandin *et al.*, 2011), and, therefore, high translocation rates. The abundances of about ten other sugars and sugar alcohols slowly increased during the mature leaf stage (S_L3a and S_L3b), reflecting a potential role for some of these specific molecules in regulating the metabolism of the mature leaf.

The close link between sugar accumulation and the induction of leaf senescence has been widely reported in the literature (Masclaux *et al.*, 2000; Yoshida, 2003; Wingler *et al.*, 2006; Wingler and Roitsch, 2008), although a decrease of sugar content during leaf senescence has been also found in species, such as sunflower (*Helianthus annuus* L.) (Moschen *et al.*, 2016). Changes in sugar levels at the onset of leaf senescence were variable, and depended upon species, treatments, and leaf section assessed (van Doorn, 2008). Amongst the metabolites with significant growth temperature × harvest time interaction effects, three of five sugars and sugar alcohols, viz., galactose (M19), glucose (M22), and mannitol (M54), were more abundant at the beginning of leaf senescence, but only at the highest temperature. These metabolites, therefore, may not act as general triggering metabolites for leaf senescence, although we cannot rule out the possibility that different metabolites are triggering senescence under different temperature regimes. We identified five metabolites that could act as potential signalling factors inducing leaf senescence, regardless of growth temperature. These included 2-O-glycerol-β-D-galactopyranoside (M02) and mannose (M55), which were associated with the S_L4, fructose

(M15) and sorbose (M72), which were associated with S_L3b, and maltose (M53; Fig 3-8C), which was associated with S3a. All increased in abundance in the leaves towards the end of the season. Interestingly, 2-O-glycerol-β-D-galactopyranoside exhibited a decrease in the bulbs towards the end of the season (Fig. 3-12B), suggesting that this sugar may be involved in the sink-to-source signalling pathway towards leaf senescence.

Increases in the abundances of these specific sugars and of the total soluble sugars took place before the occurrence of any visual sign of leaf senescence, as has been previously reported (Gandin *et al.*, 2011). Therefore, it is unlikely that their abundance is due to a decline in functional and structural integrity of cell membranes, and to the acceleration of membrane lipid catabolism, which produces sugars by gluconeogenesis (Lim *et al.*, 2007). The gradual increase of sugars that was observed in the present study would support the hypothesis of the build-up of a sink limitation through time as bulb growth slows down, eventually inducing leaf senescence (Lapointe, 2001). A sugar-dependent signal is a likely candidate that could link source activity to sink limitation. Further, carbohydrates are needed during the senescence process. In *Arabidopsis*, starch accumulates in the mature leaf and is subsequently used as the main source of energy to support the senescence process (Kim 2019). Yet, in species such as *Erythronium* that do not accumulate starch in their leaves, high soluble sugar concentrations at the onset of senescence can be necessary to sustain senescence activities.

One major event during leaf senescence is the production of reactive oxygen species (ROS) (Zimmermann and Zentgraf, 2005; Jajić *et al.*, 2015). Under physiological steady-state conditions, ROS are scavenged by different antioxidative defence components to maintain an equilibrium between production and scavenging of ROS (Apel and Hirt, 2004). As an end-product of ascorbic acid catabolism (Loewus, 1999), threonic acid-1,4-lactone (M75; Fig. 3-9F) increases at the two lower temperatures might reflect a depletion of ascorbic acid. Ascorbic acid could be oxidised to a greater extent at low temperature given that net photosynthetic rated are lower (Dong *et al.*, 2018), thereby allowing more ROS to be produced (Pintó-Marijuan and Munné-Bosch, 2014). A similar trend in variation that was much less abrupt was found in a pyridine derivative, the 3-hydroxypyridine (M03; Fig. 3-9H), which has rarely been reported in plants. Derivatives of 3-hydroxypyridine are water-soluble antioxidants with membrane-protective properties, suggesting that this

metabolite may be considered as a potential prophylactic agent against the damaging effects of aging (Voronina and Seredenin, 1988). At low temperature, ascorbic acid might complement the action of tocopherol (M76), which markedly increased in abundance a few days before the onset of leaf senescence at all three growth temperatures (Fig. 3-8F). Significant up-regulation of both ascorbic acid and tocopherol has been found during the senescence of tobacco leaves (Li *et al.*, 2016). Furthermore, we noted a positive correlation between CKs and tocopherol abundance, which suggested that CKs may be involved in regulating the antioxidant defence system. In transgenic tobacco plants expressing *isopentenyltransferase* (*IPT*, an enzyme that mediates CK synthesis), Rubio-Wilhelmi *et al.* (2011) reported that under N-deficient conditions, oxidative stress was absent in transgenic plants, which exhibited enhanced CK synthesis. A whole set of antioxidant defences might be more efficient to withstand oxidative damage than a single antioxidant (Munné-Bosch, 2005); however, the coordinated regulation of these essential antioxidants remains to be understood. Soluble sugars have been identified as another group of compounds that could act as ROS scavengers (Couée *et al.*, 2006; Bolouri-Moghaddam *et al.*, 2010). The simultaneous increase in fructose (M15), sorbose (M72) and tocopherol (M76) a few days before the onset of leaf senescence (Fig. 3-8F) bring support to this hypothesis.

3.4.2 Changes at the Bulb Level

ABA was the only phytohormone that was associated with new bulb growth (S_B3). Given that ABA promotes starch synthesis in potato (*Solanum tuberosum* L.) and tulip (*Tulipa gesneriana* L.) (Aung and De Hertogh, 1979; Borzenkova and Borovkova, 2003), it also may have stimulated massive starch accumulation that took place during this stage in *E. americanum* bulbs. ABA remained high even after the bulbs reached dormancy (T9), as has been shown during dormancy of other bulb-producing species (Yamazaki *et al.*, 2002; Naor *et al.*, 2008). GAs such as GA_4 also peaked or increased towards the end of the season, consistent with reports for dormant potato tubers, but according to Suttle (2004), GAs do not control tuber dormancy. Obata-Sasamoto and Suzuki (1979) reported high levels of endogenous CKs during the later stages of potato tuber growth. The association of zeatin with the late developmental stage in the present study, which was more obvious under the highest temperature regime, might be related to the effect of CKs on starch synthesis. A rise

in CK levels enhanced the activity of starch-synthesising enzymes to support starch deposition (Obata-Sasamoto and Suzuki, 1979).

The new bulb is quickly filled with starch (Gandin *et al.*, 2011), with final starch concentrations representing 70 to 80 % of bulb dry mass. Many metabolites exhibited a decrease most likely due in part to the dilution effect caused by the abundance of starch. Nevertheless, some organic acids [2-ketoglutaric acid (M01) and fumaric acid (M17)] and sugars [maltose (M53), melezitose (M56), and trehalose (M77)] exhibited increases over time. Several studies of *Arabidopsis* have clearly indicated fumaric acid acts as an alternative carbon sink for photosynthates, similar to starch (Chia *et al.*, 2000; Pracharoenwattana *et al.*, 2010; Zell *et al.*, 2010; Araújo *et al.*, 2011). This suggests that some specific organic acids may function as transient storage forms of fixed carbon. The presence of maltose during starch degradation at the leaf level has been reported repeatedly (Kruger and ap Rees, 1983; Neuhaus and Schulte, 1996; Lu and Sharkey, 2006), suggesting that maltose plays a critical role in starch metabolism, being the predominant form of that is carbon exported from chloroplasts at night. The accumulation of maltose in the bulbs supports the hypothesis proposed previously in *E. americanum* that starch degradation takes place along with starch synthesis once the bulb stops growing (Gandin *et al.*, 2011). Melezitose has been recently reported to be present at high levels under stress conditions (Martinelli *et al.*, 2013), and its concentration would correlate with levels of desiccation tolerance (Gechev *et al.*, 2014). Yet, no involvement in relation to sink limitation has been documented for this metabolite. Trehalose has been shown to be involved in sugar sensing, with a specific role being assigned to its precursor, trehalose-6-phosphate (Tre6P), which may serve as a signal of sucrose availability within the leaves (Griffiths *et al.*, 2016). A strong correlation between Tre6P and sucrose has been found in both source and sink organs of many species (Lunn *et al.*, 2006; Debast *et al.*, 2011; Lunn *et al.*, 2014; Figueroa and Lunn, 2016). We likewise found that trehalose abundance was positively correlated with sucrose (M74) abundance ($P < 0.001$, $R^2 = 0.12$; data not shown), which brings supports to the signalling role of Tre6P in regulating the utilisation of available carbon at the bulb level. L-proline (M42) and nicotinic acid (M59) increased in concentration within the bulbs at the beginning of leaf senescence. Although L-proline often increased as leaves

aged and was considered as a reliable indicator of leaf senescence (Wang *et al.*, 1982; Mondal *et al.*, 1985), it was abundant in bulbs only, which limits its role as a triggering signal. Of these metabolites that increase in abundance within the bulbs just before or at the onset of leaf senescence, maltose appears the most promising as a potential signalling trigger of leaf senescence. This metabolite exhibited a rise in abundance just prior to leaf senescence in both leaves and bulbs, despite the absence of starch in the leaves. Yet, we still need to confirm that the maltose present in the leaves has been translocated from the bulbs.

3.4.3 Phytohormone/Sugar Interactions

A complex interaction between sugar and hormonal signalling pathways has been described, particularly for ABA and ethylene, which are two major hormones that are closely involved in sugar signalling (León and Sheen, 2003). In many cases, there is a positive interaction between sugar and ABA signalling, and an antagonistic relationship between sugar and ethylene signalling (Zhou *et al.*, 1998; Cheng *et al.*, 2002; Hanson and Smeekens, 2009). In the present study, similar positive interactions were apparent at the leaf level between the concentrations of ABA and specific sugars and sugar alcohols [galactose, glucose, and mannitol] through time. Further, there might be a positive relationship between CK concentrations and soluble sugars concentrations in the leaves, as the plants growing under the low temperature regimes exhibited both higher CKs and higher soluble sugar concentrations than plants under higher temperatures. Although CKs might be the triggering factors inducing increased soluble sugars formation in leaves, we previously suggested that the higher level of soluble sugars in the leaves might be due to a reduced translocation of C to the sink in cool-grown plants (Dong *et al.*, 2018). The latter response would help maintain source and sink activity in balance for a longer period of time, thereby delaying leaf senescence. CKs can also promote carbohydrate uptake by sink organs or tissues by stimulating invertase and hexose transporter activities (Gan and Amasino, 1995; Roitsch and Ehneß, 2000; Haberer and Kieber, 2002); indeed, higher concentrations of CKs within the bulbs (ZR and DHZ; Annexes 2A and 2B) at the highest temperature early in the season agrees their faster accumulation of carbohydrates than at the lower temperatures (Gandin *et al.*, 2011). Furthermore, the senescence process is likely

affected by the interaction between sugars and CKs. Kudoyarova *et al.* (2018) suggested that glucose accumulation in the leaves was quite susceptible to induce senescence but only in the context of decreasing CKs concentration. The complexity of sugar-responsiveness processes makes their interactions with phytohormones more complex, and the actors involved in signalling network require further investigation.

In conclusion, different leaf and bulb developmental stages presented specific phytohormonal and metabolomic profiles that allowed us to identify potential signalling molecules that may be involved in modulating source and sink activity. Early on, fructose-6-P and glucose-6-P exhibited a transient increase in both leaves and bulbs, which most likely reflected a transient source–sink imbalance while leaf photosynthesis was high, but the new bulb was still small. Later on, increasing concentrations of many sugars and sugar alcohols in the leaves were most likely counteracted by increasing concentrations of CKs, which are known to delay leaf senescence. This response was especially true at lower temperature, where both sugars and CKs concentrations were consistently greater than at higher temperatures. The sudden increase of ACC, ABA, and SA in the leaves might well be the triggering factor that induces senescence under warmer growth conditions, but other signalling molecules are likely to be involved at cooler temperatures. The most likely candidates are 2-O-glycerol-β-D-galactopyranoside, mannose, fructose, sorbose, and maltose. Besides acting as signalling molecules, sugars can play different roles, as sources of energy, carbon transport molecules, and basic building blocks for different biosynthetic pathways, which makes their study intrinsically complex. Transcriptome studies could offer greater insights into the role of these triggering metabolites. More precise approaches that modulate phytohormone concentrations at specific leaf developmental stages would also be yet another way to expand our current understanding of potential signalling phytohormones.

3.5 Acknowledgements

This research was financially supported by a Discovery grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to LL. The authors thank Moustafa

A. Khalf for measuring proteolytic activities and soluble protein concentrations and helping with metabolite quantification and data analyses. The authors gratefully acknowledge François Larochelle for his expert technical assistance in growth chamber experiments, Gaétan Daigle for advice on statistical analyses and Dr. William F.J.Parsons for English language revision. The authors also sincerely thank all colleagues in the laboratory who participated in plant harvests: Moustafa A. Khalf, Julie Bussières, Pierre-Paul Dion, Jade Boulanger-Pelletier and Karolane Pitre.

Table 3-2. Results of two-way ANOVAs (*P* – values) testing the effect of growth temperature and harvest date of all identified phytohormones (H01 to H17) and other metabolites (M01 to M80) detected in the leaves of *E. americanum* during the growing season under three growth temperature regimes. They are sorted alphabetically, and classified into biochemical categories. Phytohormones or metabolites without any significant effects ($P < 0.01$) are displayed in italics.

Number	Name	Class	Growth temperature	Harvest time	Interaction (Temperature × time)	Phenological stage (PCA) [1]
H01	**Abscisic acid (ABA)**	**Abscisic acid**	0.196	**< 0.001**	**< 0.001**	**S_L4**
H02	**1-Aminocyclopropane-1-carboxylic acid (ACC)**	**Ethylene precursor**	**0.005**	**< 0.001**	**< 0.001**	**S_L3**
H03	**Zeatin (Z)**	**Cytokinins**	**< 0.001**	**< 0.001**	**< 0.001**	**S_L4**
H04	Zeatin riboside (ZR)	Cytokinins	< 0.001	< 0.001	< 0.001	S_L2
H05	**Dihydrozeatin (DHZ)**	**Cytokinins**	**< 0.001**	**< 0.001**	**< 0.001**	**S_L4**
H06	**Dihydrozeatin riboside (DHZR)**	**Cytokinins**	**< 0.001**	**< 0.001**	**< 0.001**	**S_L4**
H07	**2-isopentenyladenine (2-iP)**	**Cytokinins**	**< 0.001**	**< 0.001**	**< 0.001**	**S_L4**
H08	Isopentenyl adenosine (iPA)	Cytokinins	0.014	< 0.001	< 0.001	S_L2
H09	*GA_1*	*Gibberellins*	*0.640*	*0.369*	*0.086*	*S_L3*
H10	GA_4	Gibberellins	0.355	< 0.001	< 0.001	S_L2
H11	**GA_9**	**Gibberellins**	0.200	0.019	**< 0.001**	**S_L3**
H12	**GA_{19}**	**Gibberellins**	0.424	**< 0.001**	**< 0.001**	**S_L3**
H13	**GA_{20}**	**Gibberellins**	0.917	**< 0.001**	**< 0.001**	**S_L3**
H14	**GA_{24}**	**Gibberellins**	0.010	**< 0.001**	**< 0.001**	**S_L3**
H15	**Indole-3-acetic acid (IAA)**	**Auxins**	0.999	0.021	**< 0.001**	**S_L3**
H16	*Jasmonic acid (JA)*	*Jasmonic acid*	*0.603*	*0.200*	*0.273*	*N/A* [2]
H17	**Salicylic acid (SA)**	**Salicylic acid**	0.609	**< 0.001**	**< 0.001**	**S_L4**
M01	**2-Ketoglutaric acid**	**Organic acids**	**< 0.001**	**0.009**	0.012	**S_L4**
M02	**2-O-glycerol-β-D-galactopyranoside**	**Sugars**	0.039	**< 0.001**	0.024	**S_L4**
M03	**3-Hydroxypyridine**	**Others**	0.120	**< 0.001**	**0.009**	**S_L3b**
M04	4-Aminobutyric acid (GABA)	Amino acids	0.239	< 0.001	0.024	S_L2a
M05	Adenosine	Others	0.013	< 0.001	0.483	S_L2a
M06	*α-Linolenic acid*	*Fatty acids*	*0.412*	*0.011*	*0.502*	*S_L3b*
M07	Arabinose	Sugars	0.825	0.003	0.212	*N/A* [2]
M08	*Arabitol*	*Sugar alcohols*	*0.710*	*0.560*	*0.048*	*S_L4*
M09	Benzoic acid	Organic acids	0.160	< 0.001	0.523	S_L2a
M10	**β-Sitosterol**	**Others**	0.813	**< 0.001**	0.040	**S_L3a**
M11	Citramalic acid	Organic acids	0.307	< 0.001	0.150	S_L2a
M12	Citric acid	Organic acids	0.268	< 0.001	0.601	S_L2a
M13	*Erythritol*	*Sugar alcohols*	*0.860*	*0.043*	*0.052*	*S_L3b*
M14	Ethanolamine	Others	0.127	< 0.001	0.515	S_L2a
M15	**Fructose**	**Sugars**	0.933	**< 0.001**	0.364	**S_L3b**

Table 3-2. (continued)

Number	Name	Class	Growth temperature	Harvest time	Interaction (Temperature × time)	Phenological stage (PCA) [1]
M16	Fructose-6-phosphate	Sugars	0.128	< 0.001	0.084	S_L2b
M17	Fumaric acid	Organic acids	0.204	< 0.001	0.031	S_L2a
M18	Galactinol	Sugar alcohols	0.935	< 0.001	0.626	S_L2a
M19	**Galactose**	**Sugars**	0.888	**< 0.001**	**0.008**	**S_L4**
M20	Galactosylglycerol	Sugar alcohols	0.683	< 0.001	0.943	S_L2a
M21	**Gentiobiose**	**Sugars**	0.180	**< 0.001**	0.370	**S_L3a**
M22	**Glucose**	**Sugars**	0.888	**< 0.001**	**0.009**	**S_L3b**
M23	Glucose-6-phosphate	Sugars	0.356	< 0.001	0.003	S_L2b
M24	**Glyceric acid**	**Organic acids**	0.097	**< 0.001**	0.051	**S_L3a**
M25	Glycerol	Sugar alcohols	0.157	< 0.001	0.711	S_L2a
M26	Glycine	Amino acids	0.153	< 0.001	0.178	S_L2a
M27	Guanosine	Others	0.024	< 0.001	0.421	S_L2a
M28	Hexadecanoic acid (Palmitic acid)	Fatty acids	0.072	< 0.001	0.694	S_L2a
M29	Hydroxybutyric acid-lactone (Butyrolactone)	Organic acids	0.010	< 0.001	0.173	S_L2a
M30	**Isomaltose**	**Sugars**	0.261	**0.001**	0.065	**S_L3a**
M31	L-Alanine	Amino acids	0.213	< 0.001	0.033	S_L2a
M32	L-Asparagine	Amino acids	0.547	< 0.001	0.266	S_L2a
M33	L-Aspartic acid	Amino acids	0.016	< 0.001	0.071	S_L2a
M34	L-Glutamic acid	Amino acids	0.002	< 0.001	0.059	S_L2a
M35	L-Glutamine	Amino acids	0.027	< 0.001	0.006	S_L2a
M36	L-Histidine	Amino acids	0.011	< 0.001	< 0.001	S_L2a
M37	L-Isoleucine	Amino acids	0.510	< 0.001	0.026	S_L2a
M38	L-Leucine	Amino acids	0.206	< 0.001	0.118	S_L2a
M39	L-Lysine	Amino acids	0.727	< 0.001	0.024	S_L2a
M40	L-Methionine	Amino acids	0.848	< 0.001	0.280	S_L2a
M41	L-Phenylalanine	Amino acids	< 0.001	< 0.001	0.067	S_L2a
M42	L-Proline	Amino acids	0.168	< 0.001	0.112	S_L2a
M43	L-Serine	Amino acids	< 0.001	< 0.001	0.002	S_L2a
M44	L-Threonine	Amino acids	0.612	< 0.001	0.800	S_L2a
M45	L-Tryptophan	Amino acids	0.432	< 0.001	0.851	S_L2a
M46	L-Tyrosine	Amino acids	0.062	< 0.001	0.036	S_L2a
M47	L-Valine	Amino acids	0.031	< 0.001	0.090	S_L2a
M48	**Lactic acid**	**Organic acids**	0.391	**0.004**	0.015	**S_L3a**
M49	Maleic acid	Organic acids	0.271	< 0.001	0.024	S_L2a
M50	**Malic acid**	**Organic acids**	0.300	**0.007**	0.059	**S_L4**
M51	**Malonic acid**	**Organic acids**	0.738	**< 0.001**	0.072	**S_L3a**
M52	*Maltitol*	*Sugar alcohols*	*0.074*	*0.034*	*0.042*	*S_L3a*

Table 3-2. (continued)

Number	Name	Class	Growth temperature	Harvest time	Interaction (Temperature × time)	Phenological stage (PCA) [1]
M53	**Maltose**	**Sugars**	0.137	**< 0.001**	0.031	**S_L3a**
M54	**Mannitol**	**Sugar alcohols**	0.309	**< 0.001**	**0.009**	**S_L4**
M55	**Mannose**	**Sugars**	0.878	**< 0.001**	0.012	**S_L4**
M56	Melezitose	Sugars	0.530	0.007	0.067	S_L2a
M57	Melibiose	Sugars	0.993	< 0.001	0.466	S_L2a
M58	**Myo-Inositol**	**Sugar alcohols**	0.136	**< 0.001**	0.155	**S_L3a**
M59	Nicotinic acid	Organic acids	0.548	< 0.001	0.392	S_L2a
M60	Norleucine	Amino acids	0.277	< 0.001	0.075	S_L2a
M61	Norvaline	Amino acids	0.515	< 0.001	0.840	S_L2a
M62	**Octadecadienoic (Linoleic acid)**	**Fatty acids**	0.073	**< 0.001**	**< 0.001**	**S_L3b**
M63	**Octadecanoic (Stearic acid)**	**Fatty acids**	0.720	**< 0.001**	0.053	**S_L3a**
M64	Phosphoric acid	Others	0.944	< 0.001	0.840	S_L2a
M65	Propanoic acid	Organic acids	0.434	< 0.001	0.120	S_L2a
M66	Pyroglutamic acid	Amino acids	0.697	< 0.001	0.066	S_L2a
M67	Pyruvic acid	Organic acids	0.402	< 0.001	0.029	S_L2a
M68	**Raffinose**	**Sugars**	0.176	**< 0.001**	0.064	**S_L3a**
M69	*Ribose*	*Sugars*	*0.479*	*0.013*	*0.252*	*N/A* [2]
M70	Silanamine, N,N'-methanetetraylbis[1,1,1-trimethyl–	Others	0.023	< 0.001	0.025	S_L2a
M71	*Sorbitol*	*Sugar alcohols*	*0.021*	*0.085*	*0.786*	*S_L4*
M72	**Sorbose**	**Sugars**	0.954	**< 0.001**	0.415	**S_L3b**
M73	**Succinic acid**	**Organic acids**	0.282	**0.006**	0.613	**S_L3b**
M74	**Sucrose**	**Sugars**	0.098	**< 0.001**	0.042	**S_L3a**
M75	**Threonic acid-1,4-lactone**	**Organic acids**	0.041	**< 0.001**	**0.002**	**S_L4**
M76	**Tocopherol (Vitamin E)**	**Others**	0.835	**< 0.001**	0.011	**S_L4**
M77	Trehalose	Sugars	0.143	< 0.001	0.007	S_L2a
M78	Trimethylsliyl butyrate	Fatty acids	0.511	< 0.001	< 0.001	S_L2a
M79	*Xylitol*	*Sugar alcohols*	*0.380*	*0.403*	*0.094*	*S_L4*
M80	*Xylose*	*Sugars*	*0.580*	*0.118*	*0.315*	*S_L4*

Note: A false discovery rate (FDR)–adjusted criterion of $P = 0.01$ was used to take into account the number of analyses that were run (see "Data analysis" section for more details).

[1] Associated phenological stages according to PCA results (Figs. 3-3 and 3-7). Phytohormones or metabolites that were associated with the last two stages according to PCA (S_L3a, S_L3b and S_L4) and exhibited significant effect(s) are displayed in bold.

[2] Phytohormones or metabolites that were not included with any phenological stages according to PCA results (Figs. 3-3 and 3-7).

Table 3-3. Results of two-way ANOVAs (*P* – values) testing the effect of growth temperature and harvest date of all identified phytohormones (H01 to H17) and other metabolites (M01 to M97) detected in the bulbs of *E. americanum* during the growing season under three growth temperature regimes. They are sorted by alphabetically, and classified into biochemical categories. The identification numbers are the same as those used for leaf analyses (Table 3-2). M81 to M97 are detected only in *E. americanum* bulbs. Phytohormones or metabolites without any significant effects ($P < 0.01$) are displayed in italics.

Number	Name	Class	Growth temperature	Harvest time	Interaction (Temperature × time)	Phenological stage (PCA) [1]
H01	**Abscisic acid (ABA)**	**Abscisic acid**	0.165	**0.003**	0.417	**S_B3**
H02	1-Aminocyclopropane-1-carboxylic acid (ACC)	Ethylene precursor	0.660	0.001	0.002	S_B2
H03	**Zeatin (Z)**	**Cytokinins**	**< 0.001**	**< 0.001**	**< 0.001**	**S_B4**
H04	Zeatin riboside (ZR)	Cytokinins	0.411	< 0.001	0.004	S_B2
H05	Dihydrozeatin (DHZ)	Cytokinins	< 0.001	< 0.001	0.003	S_B2
H06	Dihydrozeatin riboside (DHZR)	Cytokinins	0.176	0.008	0.296	S_B2
H07	2-isopentenyladenine (2-iP)	Cytokinins	0.408	0.001	0.026	S_B2
H08	Isopentenyl adenosine (iPA)	Cytokinins	0.203	< 0.001	0.001	S_B2
H09	GA_1	Gibberellins	< 0.001	< 0.001	< 0.001	S_B2
H10	**GA_4**	**Gibberellins**	0.107	**< 0.001**	**< 0.001**	**S_B4**
H11	GA_9	Gibberellins	0.780	< 0.001	< 0.001	S_B1
H13	GA_{20}	Gibberellins	< 0.001	< 0.001	< 0.001	S_B1
H14	GA_{24}	Gibberellins	0.396	< 0.001	< 0.001	S_B2
H15	Indole-3-acetic acid (IAA)	Auxins	< 0.001	< 0.001	< 0.001	S_B2
H16	Jasmonic acid (JA)	Jasmonic acid	0.020	< 0.001	0.003	S_B2
H17	Salicylic acid (SA)	Salicylic acid	< 0.001	< 0.001	< 0.001	S_B2
M01	**2-ketoglutaric acid**	**Organic acids**	0.031	**< 0.001**	**0.005**	**S_B4**
M02	**2-O-Glycerol-β-D-galactopyranoside**	**Sugars**	0.117	**< 0.001**	**0.002**	**S_B3**
M04	**4-Aminobutyric acid (GABA)**	**Amino acids**	0.269	**< 0.001**	**< 0.001**	**S_B3**
M05	**Adenosine**	**Others**	0.016	**< 0.001**	**< 0.001**	**S_B4**
M06	α - Linolenic acid	Fatty acids	0.536	< 0.001	0.002	S_B2
M07	**Arabinose**	**Sugars**	**0.006**	**< 0.001**	**< 0.001**	**S_B3**
M09	Benzoic acid	Organic acids	< 0.001	< 0.001	0.057	S_B2
M10	β - Sitosterol	Others	0.007	< 0.001	< 0.001	S_B2
M11	Citramalic acid	Organic acids	0.003	< 0.001	0.008	S_B2
M12	**Citric acid**	**Organic acids**	0.072	**< 0.001**	**< 0.001**	**S_B4**
M13	Erythritol	Sugar alcohols	< 0.001	< 0.001	< 0.001	S_B2
M14	Ethanolamine	Others	0.007	< 0.001	< 0.001	S_B2
M15	Fructose	Sugars	0.050	< 0.001	< 0.001	S_B2
M16	Fructose-6-phosphate	Sugars	< 0.001	< 0.001	< 0.001	S_B2

Table 3-3. (continued)

Number	Name	Class	Growth temperature	Harvest time	Interaction (Temperature × time)	Phenological stage (PCA) [1]
M17	**Fumaric acid**	**Organic acids**	**0.001**	**< 0.001**	**< 0.001**	**S_B4**
M18	Galactinol	Sugar alcohols	0.819	< 0.001	0.316	S_B2
M19	**Galactose**	**Sugars**	0.281	**< 0.001**	**< 0.001**	**S_B3**
M20	**Galactosylglycerol**	**Sugar alcohols**	0.118	**< 0.001**	0.100	**S_B3**
M21	Gentiobiose	Sugars	0.015	< 0.001	< 0.001	S_B2
M22	Glucose	Sugars	0.137	< 0.001	0.006	S_B2
M23	**Glucose-6-phosphate**	**Sugars**	0.588	**0.009**	0.576	**S_B3**
M24	**Glyceric acid**	**Organic acids**	0.561	**< 0.001**	0.172	**S_B3**
M25	**Glycerol**	**Sugar alcohols**	0.029	**< 0.001**	0.064	**S_B3**
M26	Glycine	Amino acids	< 0.001	< 0.001	< 0.001	S_B2
M27	Guanosine	Others	0.003	< 0.001	< 0.001	S_B2
M28	Hexadecanoic acid (Palmitic acid)	Fatty acids	0.250	< 0.001	0.005	S_B2
M29	Hydroxybutyric acid lactone	Organic acids	0.632	< 0.001	< 0.001	S_B2
M30	Isomaltose	Sugars	0.010	< 0.001	< 0.001	S_B2
M31	L-alanine	Amino acids	0.633	< 0.001	0.746	S_B2
M32	L-asparagine	Amino acids	0.001	< 0.001	< 0.001	S_B2
M33	L-aspartic acid	Amino acids	0.436	< 0.001	< 0.001	S_B2
M34	**L-glutamic acid**	**Amino acids**	0.448	**< 0.001**	0.062	**S_B4**
M35	**L-glutamine**	**Amino acids**	0.075	**< 0.001**	**< 0.001**	**S_B4**
M36	**L-histidine**	**Amino acids**	**0.004**	**< 0.001**	**< 0.001**	**S_B4**
M37	L-isoleucine	Amino acids	< 0.001	< 0.001	< 0.001	S_B2
M38	L-leucine	Amino acids	< 0.001	< 0.001	< 0.001	S_B2
M39	L-lysine	Amino acids	< 0.001	< 0.001	< 0.001	S_B2
M40	L-methionine	Amino acids	< 0.001	< 0.001	< 0.001	S_B2
M41	L-phenylalanine	Amino acids	0.005	< 0.001	< 0.001	S_B2
M42	**L-proline**	**Amino acids**	0.204	**< 0.001**	0.030	**S_B4**
M43	L-serine	Amino acids	< 0.001	< 0.001	0.002	S_B2
M44	L-threonine	Amino acids	< 0.001	< 0.001	< 0.001	S_B2
M45	L-tryptophan	Amino acids	< 0.001	< 0.001	< 0.001	S_B2
M46	L-tyrosine	Amino acids	0.004	< 0.001	< 0.001	S_B2
M47	L-valine	Amino acids	0.002	< 0.001	< 0.001	S_B2
M48	**Lactic acid**	**Organic acids**	**0.009**	**< 0.001**	**< 0.001**	**S_B3**
M49	Maleic acid	Organic acids	0.018	< 0.001	0.385	S2
M50	Malic acid	Organic acids	0.942	< 0.001	0.004	S2
M51	Malonic acid	Organic acids	0.379	< 0.001	0.012	S2
M53	**Maltose**	**Sugars**	0.469	**< 0.001**	**< 0.001**	**S_B3**
M54	Mannitol	Sugar alcohols	0.023	< 0.001	0.127	S_B2
M55	Mannose	Sugars	0.107	< 0.001	0.005	S_B2

Table 3-3. (continued)

Number	Name	Class	Growth temperature	Harvest time	Interaction (Temperature × time)	Phenological stage (PCA) [1]
M56	**Melezitose**	**Sugars**	0.345	**< 0.001**	**< 0.001**	**S_B4**
M57	*Melibiose*	*Sugars*	*0.183*	*0.021*	*0.457*	*S_B4*
M58	Myo-Inositol	Sugar alcohols	0.003	< 0.001	< 0.001	S_B2
M59	**Nicotinic acid**	**Organic acids**	**0.009**	**0.006**	0.510	**S_B4**
M62	**Octadecadienoic (Linoleic acid)**	**Fatty acids**	0.206	**< 0.001**	0.155	**S_B3**
M63	Octadecanoic (Stearic acid)	Fatty acids	0.550	< 0.001	< 0.001	S_B2
M64	Phosphoric acid	Others	0.002	< 0.001	0.141	S_B2
M65	Propanoic acid	Organic acids	0.044	< 0.001	0.124	S_B2
M66	Pyroglutamic acid	Amino acids	< 0.001	< 0.001	0.009	S_B2
M67	**Pyruvic acid**	**Organic acids**	**0.001**	**< 0.001**	**< 0.001**	**S_B3**
M68	Raffinose	Sugars	0.015	< 0.001	0.519	S_B2
M69	**Ribose**	**Sugars**	**0.001**	**< 0.001**	**< 0.001**	**S_B3**
M71	Sorbitol	Sugar alcohols	0.023	< 0.001	0.086	S_B2
M72	Sorbose	Sugars	0.048	< 0.001	< 0.001	S_B2
M73	**Succinic acid**	**Organic acids**	**0.001**	**< 0.001**	**< 0.001**	**S_B3**
M74	Sucrose	Sugars	< 0.001	< 0.001	< 0.001	S_B2
M76	Tocopherol	Others	< 0.001	< 0.001	0.003	S_B2
M77	**Trehalose**	**Sugars**	0.014	**< 0.001**	0.024	**S_B3**
M78	Trimethylsliyl butyrate	Organic acids	0.825	< 0.001	0.003	S_B2
M80	Xylose	Sugars	0.024	< 0.001	0.124	S_B2
M81	3-Deoxy-arabino-hexaric acid	Organic acids	< 0.001	< 0.001	< 0.001	S_B2
M82	Acetic acid	Organic acids	0.273	< 0.001	< 0.001	S_B2
M83	Arabinaric acid	Organic acids	< 0.001	< 0.001	< 0.001	S_B2
M84	β - alanine	Amino acids	0.143	< 0.001	< 0.001	S_B2
M85	**Butyric acid**	**Organic acids**	**< 0.001**	**< 0.001**	**< 0.001**	**S_B3**
M86	**Gluconic acid**	**Organic acids**	0.428	**0.002**	0.290	**S_B3**
M87	Glucopyranose	Sugars	< 0.001	< 0.001	< 0.001	S_B2
M88	Glucouronic acid	Organic acids	0.145	< 0.001	0.028	S_B2
M89	**Methylene succinic acid**	**Organic acids**	**< 0.001**	**< 0.001**	0.066	**S_B3**
M90	Myo-inositol-2-phosphate	Sugar alcohols	< 0.001	< 0.001	0.103	S_B2
M91	N-acetyl glucosamine	Others	< 0.001	< 0.001	< 0.001	S_B2
M92	N-acetyl glutamic acid	Amino acids	0.037	< 0.001	0.012	S_B2
M93	**Nonanoic acid**	**Organic acids**	0.051	**< 0.001**	0.100	**S_B3**
M94	**Oleic acid**	**Fatty acids**	**< 0.001**	**< 0.001**	0.143	**S_B3**
M95	Rhamnose	Sugars	0.013	< 0.001	0.017	S_B2
M96	**Uracil**	**Others**	0.374	**< 0.001**	0.441	**S_B3**
M97	**Urea**	**Others**	0.031	**< 0.001**	0.067	**S_B3**

Note: A false discovery rate (FDR)–adjusted criterion of $P = 0.01$ was used to take into account the number of analyses that were run (see "Data analysis" section for more details).

[1] Associated phenological stages according to PCA results (Figs. 3-5 and 3-10). Phytohormones or metabolites that were associated with the last two stages according to PCA (S_B3 and S_B4) and presented significant effect(s) are displayed in bold.

Conclusion générale

Communes dans les forêts décidues d'Amérique du Nord, particulièrement dans les érablières, les érythrones d'Amérique qui apparaissent peu après la fonte des neiges indiquent le changement de saison dans le sud du Québec. Ce sont des plantes de pleine lumière (héliophyte), qui complètent leur cycle de vie avant que les arbres les plongent dans l'ombre. Des études antérieures ont montré que la croissance chez plusieurs éphémères printanières, y compris l'érythrone, est limitée par le puits (Lapointe, 2001; Badri *et al.*, 2007; Lundmark *et al.*, 2009; Gandin *et al.*, 2011; Bernatchez and Lapointe, 2012). Aux basses températures, l'activité des puits serait prolongée, ce qui retarderait la sénescence foliaire et permettrait au bulbe d'accumuler plus de biomasse (Lapointe, 2001).

Nous avons analysé certaines des activités métaboliques pouvant réguler la force du puits dans les situations de déséquilibre source–puits induites par différentes températures de croissance. Les situations de déséquilibre source–puits peuvent être transitoires, car la plante parvient souvent à ajuster l'activité des puits à celle des sources et vice versa. Si le rétablissement de l'équilibre n'est pas possible, la sénescence foliaire est induite. Nous avons également tenté d'identifier des possibles déterminants signalétiques qui pourraient apparaître avant le premier signe visible de la sénescence et déclencher ou induire la sénescence foliaire, ou des indicateurs qui apparaissent juste avant ou au moment de la sénescence et peuvent être considérés comme des outils de mesure de ce processus. Cette étude visait ainsi à approfondir le lien entre induction de la sénescence foliaire et activité du puits (bulbe).

Induction de la senescence foliaire

La croissance annuelle des érythrones a été suivie jusqu'à la sénescence foliaire. L'induction de la sénescence foliaire correspondait à l'apparition du premier signe visuel de dégradation des chlorophylles (indice d'un début de remobilisation de l'azote foliaire). Ce début de remobilisation de l'azote a été confirmé par les fortes activités protéolytiques que nous avons observées au début de la sénescence foliaire ainsi que par les teneurs en acides aminés libres qui n'augmentent pas avant le début de la sénescence (**Chapitre 3**). Une augmentation de la capacité respiratoire (taux de respiration totale, R_T) permet de

compenser totalement (homéostasie) l'impact des températures froides sur les taux respiratoires (**Chapitre 1**). En parallèle à cette acclimatation à basse température du R_T de la feuille, une réduction de l'assimilation (A) a conduit à un quotient R_T/A plus élevé, reflétant probablement une réduction de la quantité de carbone (C) exporté vers le bulbe (Figure V). Les teneurs en sucres solubles foliaires étaient plus élevées à 8 °C qu'aux autres températures (Figure 1-5, Tableau 1-1, et Figure 3-1) ce qui suggère un effet de la température sur les mécanismes de chargement du phloème (anatomie des veines ou activité des transporteurs) ou sur le taux de transport de la sève phloémienne (viscosité). De plus, comme les taux respiratoires que nous avons mesurés étaient complètement dépendants des niveaux de substrats endogènes (Atkin and Tjoelker, 2003), les changements dans la respiration et les concentrations de sucres solubles au niveau de la feuille étaient tel qu'attendu liés les uns aux autres.

Dans cette étude, l'acclimatation de la voie respiratoire alternative (R_{alt}) a été observée dans la feuille, mais n'a pas été fortement stimulée. Néanmoins, son importance par rapport à la respiration totale était évidente à basse température: les rapports entre R_{alt} et R_T étaient significativement plus élevés à 8/6 °C tout au long de la saison par rapport aux autres régimes de température (données non présentées). Nous n'avons pas pu, dans la présente étude, présenter les activités de ces deux voies respiratoires ni la participation de la voie alternative du fait que la voie cytochromique n'est pas nécessairement toujours saturée et que les deux voies respiratoires peuvent entrer en compétition pour les électrons. Il est maintenant largement admis que la seule technique fiable pour étudier le partitionnement des électrons (c'est-à-dire l'activité) entre la voie alternative et la voie cytochromique consiste à utiliser le fractionnement isotopique de l'oxygène (Day *et al.*, 1996; Ribas-Carbo *et al.*, 2005). Bien que nos mesures à l'aide d'une électrode d'oxygène ne soient pas les plus appropriées pour les mesures des activités des voies respiratoires, notre estimation de l'activité R_{alt} nous a conduit à la question suivante: est-ce que la voie alternative pourrait être impliquée dans la production de la chaleur chez l'érythrone ? Effectivement, cette voie respiratoire est connue pour être responsable de la thermogenèse des organes floraux chez certaines espèces (Wagner *et al.*, 1998). Pour ce faire, nous avons mesuré les températures foliaires à l'aide d'une caméra infra-rouge (analyses non présentées), mais les résultats

obtenus n'ont pas montré de différence thermique significative feuille-air entre les trois températures de croissance. Les feuilles sont des structures très fines, ayant un rapport surface/volume très élevé ce qui favorise les échanges avec l'environnement et dissipe une bonne partie du gradient de température entre l'air et la feuille. Cela pourrait possiblement expliquer l'absence d'un réchauffement au niveau de la feuille. De plus, la ventilation continue dans les chambres de croissance pourrait aussi contribuer à atténuer l'effet de réchauffement au niveau de la feuille. Par contre, ce phénomène pourrait être présent en conditions naturelles.

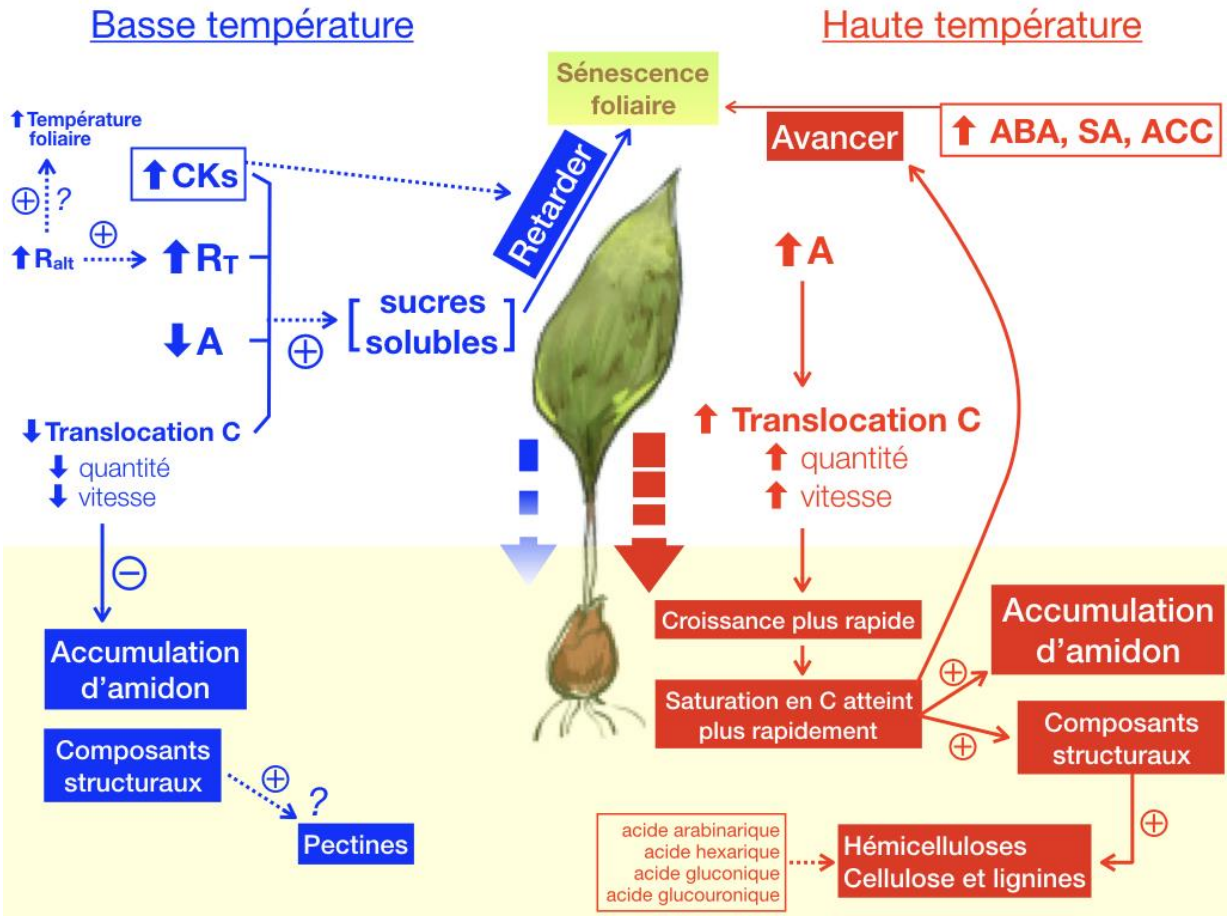


Figure V. Schéma synthétique résumant les principaux résultats et hypothèses concernant la régulation de l'activité du puits et l'induction de la sénescence foliaire chez l'érythrone d'Amérique en fonction de sa température de croissance. Les traits pleins indiquent les relations testées dans la présente étude. Les traits pointillés indiquent des hypothèses ou des propositions discutées qui nécessiteront d'autres études.

En parallèle à l'accumulation des sucres dans la feuille, une augmentation des concentrations en cytokinines (CKs) dans la feuille mature révèle probablement leur lien étroit avec des sucres. Les teneurs élevées en CKs sont connues pour retarder la sénescence foliaire, mais les mécanismes ne sont pas bien élucidés (Zwack and Rashotte, 2013; Edlund *et al.*, 2017). Il est généralement reconnu que leur influence est partiellement exercée par la stimulation de l'activité de l'invertase extracellulaire (invertase de la paroi cellulaire) (Roitsch and Ehneß, 2000; Balibrea Lara *et al.*, 2004; Zwack and Rashotte, 2013). L'augmentation de l'activité de cette enzyme clé est très probablement susceptible de perturber le chargement apoplastique du phloème par l'induction des transporteurs d'hexoses et l'hydrolyse du saccharose effluée, pour aboutir à la génération d'un « cycle futile » saccharose-hexose-saccharose (Zwack and Rashotte, 2013). Ce cycle semble permettre d'atténuer l'effet de la rétro-inhibition de la photosynthèse et éventuellement de retarder la sénescence foliaire (Figure V). Ceci souligne un autre aspect de l'induction de la sénescence foliaire qui reste toujours mal compris et parfois controversé: l'accumulation et l'abaissement des teneurs en sucres semblent tous deux déclencher la sénescence foliaire dans certaines conditions. Certains ont suggéré que des changements dans le flux de C plutôt que les teneurs en sucres pourraient stimuler l'apparition de la sénescence (Zwack and Rashotte, 2013). En outre, il faut également prendre en compte que la pertinence métabolique des sucres dépend fortement de leur distribution spatiale à la fois entre les cellules et à l'intérieur de celles-ci (Zwack and Rashotte, 2013). Cependant, ces différences ne peuvent pas être déterminées lors d'expériences de mesure des sucres à partir d'un extrait de feuille entière.

Le lien entre l'induction de la sénescence foliaire et les teneurs en sucres n'est jamais simple, mais certains sucres et leurs dérivés semblent être les composés les plus susceptibles de lier l'activité de la source à celle du puits. L'accroissement transitoire des sucres phosphorylés en début de saison pourrait indiquer un déséquilibre source–puits momentané, où la photosynthèse est élevée au niveau de la feuille mais le nouveau bulbe encore petit. Nous avons aussi remarqué que l'abondance de nombreux sucres et polyols dans la feuille a augmenté vers la fin de saison, juste avant le début de la sénescence foliaire, à savoir le 2-O-glycérol-β-D-galactopyranoside, le fructose, le maltose, le mannose

et le sorbose (Figures 3-8C, F, et 3-12B). Malheureusement, au mieux de nos connaissances, leurs rôles potentiels comme régulateurs de l'induction de la sénescence foliaire n'ont pas été bien précisés dans la littérature. L'approche métabolomique que nous avons utilisée ne permet pas de quantifier tous les métabolites, sans compter qu'il y a encore beaucoup de pics détectés mais inconnus dans les spectres de masse. D'autres métabolites pourraient donc être impliqués dans l'induction de la sénescence foliaire.

De façon assez surprenante, la température a eu peu d'influence sur le profil métabolomique. Ainsi, nous n'avons pu voir d'impact au niveau métabolomique du processus d'acclimatation de R_T observée à basse température. Contrairement à ce qui était observé à basse température, aucune acclimatation de la respiration n'était observée chez les plantes croissant à haute température. L'abondance observée uniquement à la température la plus élevée de certaines phytohormones au début de la sénescence foliaire, à savoir l'acide abscissique (ABA), l'acide 1-aminocyclopropane-1-carboxylique (ACC, précurseur direct de l'éthylène) et l'acide salicylique (SA), suggère que ces phytohormones pourraient très probablement servir en tant que déclencheurs de la sénescence foliaire à cette haute température (Figure V).

Bien que la sénescence foliaire plus précoce soit distincte à haute température, il n'est pas tout à fait évident si le déséquilibre source–puits est plus prononcé à plus haute température de croissance. L'éthylène, l'ABA et le SA ont été signalés comme étant largement impliqués dans la réponse à divers stress biotiques et abiotiques (Lim *et al.*, 2007; Thomas, 2013). Ces stress pourraient éventuellement déclencher l'expression de gènes sensibles au stress, qui à leur tour semblent affecter la sénescence foliaire. Dans la présente étude, nous n'avons pas pu confirmer s'il existait un signe de stress, ou bien si la plus haute température (18 °C) pourrait déjà être considérée comme une température stressante pour l'érythrone. Tâchons aussi de nous rappeler que les plantes ont été récoltées plus fréquemment à température plus élevée (chaque 2, 3 et 4 jours à 18 °C, 12 °C et 8 °C, respectivement), et que nos résultats ont été rapportés en fonction des récoltes et non en fonction des jours. Des changements métaboliques similaires entre les récoltes signifient en fait des changements plus rapides à la température plus élevée qu'à la température plus

basse. Si on accepte l'hypothèse de Zwack and Rashotte (2013), les changements dans les flux de C pourraient se produire plus rapidement aux températures élevées et ainsi entrainer une signalisation phytohormonale plus marquée conduisant à une sénescence hâtive. De plus, certaines phytohormones impliquées dans le processus de sénescence foliaire, comme les brassinostéroïdes ou les strigolactones, n'ont pas été analysées dans cette étude et cela justifierait évidemment des investigations supplémentaires.

Activité du puits

Dans l'analyse métabolomique, il est intéressant de remarquer que certains acides organiques, qui sont uniquement présents dans le bulbe, semblent être des intermédiaires importants dans la voie biosynthétique des composants de la paroi cellulaire, à savoir l'acide arabinarique, l'acide hexarique, l'acide gluconique et l'acide glucouronique. Par exemple, l'acide arabinarique est un dérivé de l'arabinose, et ce dernier est le monosaccharide le plus abondant des hémicelluloses (Tableau 2-3). L'acide hexarique est un produit du métabolisme des acides uroniques, et ces derniers sont les principaux constituants des pectines. Pourtant, il est difficile de préciser leurs interactions dans les voies biosynthétiques, du fait que l'équilibre entre la synthèse et l'utilisation reste mal compris.

Chez les plantes cultivées à la température plus élevée, l'amidon s'accumule plus rapidement dans le bulbe et la taille finale du bulbe est atteinte plus vite par rapport à ce qui est observé chez les plantes cultivées à plus basse température (Gandin *et al.*, 2011). Une fois les cellules remplies d'amidon, un changement de la répartition de C dans le bulbe pourrait par ailleurs aider ces plantes à maintenir la force de leur puits et ainsi améliorer l'équilibre source−puits. Au cours de la présente étude, nous avons pu, en excluant l'effet de dilution causé par l'abondance d'amidon, remarquer un investissement de C s'accroissant avec la température de croissance vers les composants de la paroi cellulaire du bulbe, à la fois dans la fraction « cellulose et lignines » et dans la fraction hémicelluloses (Figure V). Nous avons aussi suivi les variations de ces deux fractions au cours du temps en utilisant la méthode gravimétrique de (Schädel *et al.*, 2010a), et une augmentation des

teneurs en hémicelluloses sur une base exempte d'amidon n'apparait qu'en fin de saison à haute température (données non présentées). Cela permet d'expliquer pourquoi nous avons concentré notre attention sur le dernier stade phénologique pour l'analyse de répartition de C.

Différentes destinations du C dans la paroi cellulaire pourraient mener à des conséquences opposées. Une répartition élevée de C vers la fraction « cellulose et lignines » pourrait entraîner des impacts néfastes à cause i) du rôle restrictif que jouent les lignines dans l'élongation cellulaire et ii) de leur coût de construction substantiel (Poorter and Villar, 1997), ce qui en conséquence supposerait des pertes respiratoires plus importantes et donc une influence sur le bilan de C. Au contraire, un investissement en faveur des hémicelluloses se déroule conformément à nos attentes. À la différence de la cellulose et des lignines, les hémicelluloses peuvent être recyclés et utilisés comme des réserves de C pendant des périodes de forte demande mais de fourniture réduite en C par la photosynthèse, par exemple lors du débourrement au printemps (Schädel *et al.*, 2009). Leur fonction de réserve, en plus de leur rôle primordial structural, a été bien démontrée chez plusieurs espèces, telles que certains conifères et feuillus (Robakidze and Bobkova, 2003; Hoch, 2007; Schädel *et al.*, 2009). Nous avons ainsi pu proposer que la répartition de C préférentielle aux composants de la paroi cellulaire du bulbe, spécifiquement vers les hémicelluloses, permet aux plantes cultivées à des températures plus élevées de maintenir (temporairement) la force de leur puits lorsque les cellules sont déjà remplies d'amidon.

Nous notons également un investissement du C relativement important à basse température dans d'autres composés du bulbe, qui n'ont pas été déterminés dans la présente étude et qui sont contenus dans la fraction restante non mesurée (« remaining »). Selon nos hypothèses, les pectines seraient susceptibles de faire partie de ce pool non caractérisé du fait de leur fonction de réserve supplémentaire. En effet, cette fonction a été rapportée dans les cotylédons et les graines de certaines espèces (Crawshaw and Reid, 1984; Gomez *et al.*, 2009); de plus, une augmentation de la teneur en pectines lors d'une acclimatation au froid a été observée chez diverses plantes (Weiser *et al.*, 1990; Kubacka-Zębalska and Kacperska, 1999; Solecka *et al.*, 2008; Baldwin *et al.*, 2014; Le Gall *et al.*, 2015).

Cependant, l'effet de la température sur la teneur en pectines dans les organes de réserve n'a pas encore été concrètement caractérisé dans la littérature. Est-ce que cette répartition du C vers la fraction pectines pourrait constituer une stratégie additionnelle pour le bulbe afin de moduler la force du puits chez les plantes cultivées à basse température ? Les pectines correspondent à un ensemble complexe de macromolécules constituées d'une chaîne principale et de chaînes secondaires branchées. La chaîne principale est constituée d'acide galacturonique. À l'inverse des lignines, la biosynthèse des acides organiques a un coût de construction identique à celui des sucres (Poorter and Villar, 1997). Ce coût moindre pourrait expliquer la longévité du bulbe à basse température. Des expériences sont nécessaires pour le vérifier.

Il serait très intéressant de savoir si, au niveau du bulbe, cette abondance des hémicelluloses (haute température), et probablement des pectines (basse température), pourraient être remobilisées pour la croissance au printemps suivant. Dans cette étude, l'abondance de plusieurs des acides organiques, surtout ceux qui sont fort probablement impliqués dans la voie biosynthétique des composants structuraux comme nous l'avons mentionné auparavant, était élevée en début de saison puis a diminué avec le temps (Annexe 7F et H). Cela pourrait refléter une dégradation partielle des hémicelluloses ou pectines, et suggère donc leur remobilisation dans les tissus végétaux.

Durant cette thèse, nous avons dosé l'amidon au niveau du bulbe en utilisant différentes méthodes de solubilisation et d'hydrolyse: l'eau bouillante et l'amyloglucosidase, l'alcool chaud et l'α-amylase, et une base forte suivi de l'ajout des deux enzymes (l'α-amylase et l'amyloglucosidase). Les méthodes de dosages étaient également variées : anthrone, PAHBAH, HPLC et dosage enzymatique (péroxydase-glucose oxydase/o-dianisidine). Ces procédures nous ont donné des résultats différents de teneurs en amidon. D'après Gomez *et al.* (2003) et Landhäusser *et al.* (2018), d'autres composés sont digérés et libérés en même temps que l'amidon par l'amyloglucosidase de même que durant la solubilisation à l'aide d'une base forte; ces composés sont ensuite dosés en tant que sucres réducteurs ce qui surévalue les teneurs en amidon. Certains des résultats présentés par ces auteurs suggèrent que des composés structuraux seraient ainsi

partiellement digérés, ce qui appuie nos résultats d'hémicelluloses et notre proposition concernant les pectines qui s'accumulent en fin de saison. Des procédures de dosage améliorées et plus spécifiques sont donc nécessaires pour confirmer ces hypothèses.

Conséquences écologiques

À haute température de croissance, une sénescence foliaire plus hâtive, combinée à la production d'un plus petit organe de réserve et donc à une quantité d'amidon moins importante pourraient conduire à des impacts néfastes sur la croissance de la nouvelle feuille au printemps suivant. Selon les scénarios actuels (IPCC, 2018), les prévisions de réchauffement climatique pourraient ainsi, au fil du temps, mener à la disparition de cette éphémère printanière. La composante inconnue dans ce scénario est l'impact du changement climatique sur la phénologie des arbres dominants des forêts par rapport à celle des éphémères printanières. Le débourrement chez les éphémères est lié essentiellement à la disparation de la neige, alors que celui des arbres, entre autres les érables, est modulé de façon plus complexe par les températures hivernales et printanières (Raulier and Bernier, 2000). Ainsi, il est possible que la fenêtre de temps où les éphémères peuvent profiter de la pleine lumière au printemps, raccourcisse au cours des prochaines décennies réduisant d'autant la période de photosynthèse chez les éphémères. La quantité de neige qui s'accumule au sol pourrait aussi influencer la phénologie des éphémères au printemps ce qui rend les prédictions hasardeuses pour le moment.

Cette étude ouvre la voie à des études plus spécifiques sur les différents composés qui semblent jouer un rôle dans les relations source–puits et dans l'induction de la sénescence foliaire chez les éphémères printanières. Elle nous a tout de même permis de mieux comprendre leurs adaptations physiologiques dans le contexte du changement climatique.

Bibliographie

Ahmed, N., Maekawa, M., Tetlow, I.J., 2008. Effects of low temperature on grain filling, amylose content, and activity of starch biosynthesis enzymes in endosperm of basmati rice. Australian Journal of Agricultural Research 59(7): 599-604.

Ahmed, N., Tetlow, I.J., Nawaz, S., Iqbal, A., Mubin, M., Nawaz ul Rehman, M.S., Butt, A., Lightfoot, D.A., Maekawa, M., 2015. Effect of high temperature on grain filling period, yield, amylose content and activity of starch biosynthesis enzymes in endosperm of basmati rice. Journal of the Science of Food and Agriculture 95(11): 2237-2243.

Ainsworth, E.A., Bush, D.R., 2011. Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity. Plant Physiology 155(1): 64-69.

Amor, Y., Haigler, C.H., Johnson, S., Wainscott, M., Delmer, D.P., 1995. A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. Proceedings of the National Academy of Sciences of the United States of America 92(20): 9353-9357.

Andrews, P., Hough, L., Jones, J., 1956. Mannose-containing polysaccharides. Part IV. The glucomannans of lily bulbs. Journal of the Chemical Society (Resumed): 181-188.

Apel, K., Hirt, H., 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55: 373-399.

Araújo, W.L., Nunes-Nesi, A., Fernie, A.R., 2011. Fumarate: Multiple functions of a simple metabolite. Phytochemistry 72(9): 838-843.

Armstrong, A.F., Logan, D.C., Atkin, O.K., 2006. On the developmental dependence of leaf respiration: responses to short-and long-term changes in growth temperature. American Journal of Botany 93(11): 1633-1639.

Arnone III, J.A., Körner, C., 1997. Temperature adaptation and acclimation potential of leaf dark respiration in two species of *Ranunculus* from warm and cold habitats. Arctic and Alpine Research 29(1): 122-125.

Arrom, L., Munné-Bosch, S., 2012. Hormonal regulation of leaf senescence in *Lilium*. Journal of Plant Physiology 169(15): 1542-1550.

Asano, T., Kunieda, N., Omura, Y., Ibe, H., Kawasaki, T., Takano, M., Sato, M., Furuhashi, H., Mujin, T., Takaiwa, F., Wu, C.-y., Tada, Y., Satozawa, T., Sakamoto, M., Shimada, H., 2002. Rice SPK, a calmodulin-like domain protein kinase, is required for storage product accumulation during seed development: Phosphorylation of sucrose synthase is a possible factor. Plant Cell 14(3): 619-628.

Atkin, O.K., Bruhn, D., Hurry, V.M., Tjoelker, M.G., 2005a. The hot and the cold: unravelling the variable response of plant respiration to temperature. Functional Plant Biology 32(2): 87-105.

Atkin, O.K., Bruhn, D., Tjoelker, M.G., 2005b. Response of plant respiration to changes in temperature: mechanisms and consequences of variations in Q_{10} values and acclimation. *In*: Plant Respiration. *Edited by* Lambers, H., Ribas-Carbo, M., Springer, pp. 95-135.

Atkin, O.K., Cummins, W.R., 1994. The effect of nitrogen source on growth, nitrogen economy and respiration of two high arctic plant species differing in relative growth rate. Functional Ecology 8(3): 389-399.

Atkin, O.K., Edwards, E.J., Loveys, B.R., 2000a. Response of root respiration to changes in temperature and its relevance to global warming. New Phytologist 147(1): 141-154.

Atkin, O.K., Holly, C., Ball, M., 2000b. Acclimation of snow gum (*Eucalyptus pauciflora*) leaf respiration to seasonal and diurnal variations in temperature: the importance of changes in the capacity and temperature sensitivity of respiration. Plant, Cell & Environment 23(1): 15-26.

Atkin, O.K., Tjoelker, M.G., 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. Trends in Plant Science 8(7): 343-351.

Aung, L.H., De Hertogh, A.A., 1979. Temperature regulation of growth and endogenous abscisic acid-like content of *Tulipa gesneriana* L. Plant Physiology 63(6): 1111-1116.

Azcon-Bieto, J., Lambers, H., Day, D.A., 1983. Respiratory properties of developing bean and pea leaves. Functional Plant Biology 10(3): 237-245.

Badri, M.A., Minchin, P.E.H., Lapointe, L., 2007. Effects of temperature on the growth of spring ephemerals: *Crocus vernus*. Physiologia Plantarum 130(1): 67-76.

Baldwin, L., Domon, J.M., Klimek, J.F., Fournet, F., Sellier, H., Gillet, F., Pelloux, J., Lejeune-Henaut, I., Carpita, N.C., Rayon, C., 2014. Structural alteration of cell wall pectins accompanies pea development in response to cold. Phytochemistry 104: 37-47.

Balibrea Lara, M.E., Gonzalez Garcia, M.-C., Fatima, T., Ehneß, R., Lee, T.K., Proels, R., Tanner, W., Roitsch, T., 2004. Extracellular invertase is an essential component of cytokinin-mediated delay of senescence. The Plant Cell 16(5): 1276-1287.

Baroja-Fernández, E., Muñoz, F.J., Li, J., Bahaji, A., Almagro, G., Montero, M., Etxeberria, E., Hidalgo, M., Sesma, M.T., Pozueta-Romero, J., 2012. Sucrose synthase activity in the *sus1/sus2/sus3/sus4 Arabidopsis* mutant is sufficient to support normal cellulose and starch production. Proceedings of the National Academy of Sciences, USA 109(1): 321-326.

Barratt, D.H.P., Derbyshire, P., Findlay, K., Pike, M., Wellner, N., Lunn, J., Feil, R., Simpson, C., Maule, A.J., Smith, A.M., 2009. Normal growth of *Arabidopsis* requires cytosolic invertase but not sucrose synthase. Proceedings of the National Academy of Sciences, USA 106(31): 13124-13129.

Barth, C., De Tullio, M., Conklin, P.L., 2006. The role of ascorbic acid in the control of flowering time and the onset of senescence. Journal of Experimental Botany 57(8): 1657-1665.

Basu, P., Sharma, A., Garg, I., Sukumaran, N., 1999. Tuber sink modifies photosynthetic response in potato under water stress. Environmental and Experimental Botany 42(1): 25-39.

Bernatchez, A., Lapointe, L., 2012. Cooler temperatures favour growth of wild leek (*Allium tricoccum*), a deciduous forest spring ephemeral. Botany 90(11): 1125-1132.

Berry, J., Bjorkman, O., 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology 31(1): 491-543.

Blakeney, A.B., Mutton, L.L., 1980. A simple colorimetric method for the determination of sugars in fruit and vegetables Journal of the Science of Food and Agriculture 31(9): 889-897.

Blodgett, F.H., 1910. The origin and development of bulbs in the genus *Erythronium*. Botanical Gazette 50(5): 340-373.

Bolouri-Moghaddam, M.R., Le Roy, K., Xiang, L., Rolland, F., Van den Ende, W., 2010. Sugar signalling and antioxidant network connections in plant cells. The FEBS Journal 277(9): 2022-2037.

Bonnier, F.J., Hoekstra, F.A., De Vos, C.R., Van Tuyl, J.M., 1997. Viability loss and oxidative stress in lily bulbs during long-term cold storage. Plant Science 122(2): 133-140.

Borzenkova, R., Borovkova, M., 2003. Developmental patterns of phytohormone content in the cortex and pith of potato tubers as related to their growth and starch content. Russian Journal of Plant Physiology 50(1): 119-124.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Braun, D.M., Wang, L., Ruan, Y.-L., 2014. Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security. Journal of Experimental Botany 65(7): 1713-1735.

Buckeridge, M.S., dos Santos, H.P., Tiné, M.A.S., 2000. Mobilisation of storage cell wall polysaccharides in seeds. Plant Physiology and Biochemistry 38(1): 141-156.

Buckeridge, M.S., Rayon, C., Urbanowicz, B., Tiné, M.A.S., Carpita, N.C., 2004. Mixed linkage (1→3),(1→4)-β-d-glucans of grasses. Cereal Chemistry Journal 81(1): 115-127.

Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O., Hurry, V., 2007. Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. New Phytologist 176(2): 375-389.

Chapin, F.S., Schulze, E.-D., Mooney, H.A., 1990. The ecology and economics of storage in plants. Annual Review of Ecology and Systematics 21: 423-447.

Cheng, W.-H., Endo, A., Zhou, L., Penney, J., Chen, H.-C., Arroyo, A., Leon, P., Nambara, E., Asami, T., Seo, M., Koshiba, T., Sheen, J., 2002. A unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. The Plant Cell 14(11): 2723-2743.

Chia, D.W., Yoder, T.J., Reiter, W.D., Gibson, S.I., 2000. Fumaric acid: an overlooked form of fixed carbon in *Arabidopsis* and other plant species. Planta 211(5): 743-751.

Chourey, P.S., Taliercio, E.W., Carlson, S.J., Ruan, Y.-L., 1998. Genetic evidence that the two isozymes of sucrose synthase present in developing maize endosperm are critical, one for cell wall integrity and the other for starch biosynthesis. Molecular and General Genetics MGG 259(1): 88-96.

Claassens, M.M.J., 2002. Carbohydrate metabolism during potato tuber dormancy and sprouting. Department of Plant Sciences, Laboratory of Plant Physiology. Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands, p. 144.

Collier, D.E., 1996. No difference in leaf respiration rates among temperate, subarctic, and arctic species grown under controlled conditions. Canadian Journal of Botany 74(2): 317-320.

Collier, D.E., Cummins, W.R., 1990. The effects of low growth and measurement temperature on the respiratory properties of five temperate species. Annals of Botany 65(5): 533-538.

Contento, A.L., Kim, S.-J., Bassham, D.C., 2004. Transcriptome profiling of the response of Arabidopsis suspension culture cells to Suc starvation. Plant Physiology 135(4): 2330-2347.

Cosgrove, D.J., 2005. Growth of the plant cell wall. Nature Reviews Molecular Cell Biology 6(11): 850-861.

Coué, I., Sulmon, C., Gouesbet, G., El Amrani, A., 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. Journal of Experimental Botany 57(3): 449-459.

Crawshaw, L.A., Reid, J.S.G., 1984. Changes in cell-wall polysaccharides in relation to seedling development and the mobilisation of reserves in the cotyledons of *Lupinus angustifolius* cv. Unicrop. Planta 160(5): 449-454.

Day, D.A., Krab, K., Lambers, H., Moore, A.L., Siedow, J.N., Wagner, A.M., Wiskich, J.T., 1996. The cyanide-resistant oxidase: to inhibit or not to inhibit, that is the question. Plant Physiology 110(1): 1.

De Hertogh, A., Le Nard, M., 1993. The physiology of flower bulbs. A comprehensive treatise on the physiology and utilization of ornamental flowering bulbous and tuberous plants. Elsevier Science Publishers B.V., Amsterdam, Netherlands.

Debast, S., Nunes-Nesi, A., Hajirezaei, M.R., Hofmann, J., Sonnewald, U., Fernie, A.R., Börnke, F., 2011. Altering trehalose-6-phosphate content in transgenic potato tubers affects tuber growth and alters responsiveness to hormones during sprouting. Plant Physiology 156(4): 1754-1771.

DeJong, T.M., Grossman, Y.L., 1995. Quantifying sink and source limitations on dry matter partitioning to fruit growth in peach trees. Physiologia Plantarum 95(3): 437-443.

Dewar, R.C., Medlyn, B.E., Mcmurtrie, R., 1999. Acclimation of the respiration/photosynthesis ratio to temperature: insights from a model. Global Change Biology 5(5): 615-622.

Diaz, C., Lemaitre, T., Christ, A., Azzopardi, M., Kato, Y., Sato, F., Morot-Gaudry, J.F., Le Dily, F., Masclaux-Daubresse, C., 2008. Nitrogen recycling and remobilization are differentially controlled by leaf senescence and development stage in *Arabidopsis* under low nitrogen nutrition. Plant Physiology 147(3): 1437-1449.

Diaz, C., Purdy, S., Christ, A., Morot-Gaudry, J.F., Wingler, A., Masclaux-Daubresse, C., 2005. Characterization of markers to determine the extent and variability of leaf senescence in Arabidopsis. A metabolic profiling approach. Plant Physiology 138(2): 898-908.

Dickson, R.E., 1991. Assimilate distribution and storage. *In*: Physiology of Trees. *Edited by* Raghavendra, A.S., John Wiley and Sons, New York, pp. 51-85.

Dong, Y.W., Gérant, D., Lapointe, L., 2018. Thermal acclimation of leaf respiration as a way to reduce source-sink imbalance at low temperatures in *Erythronium americanum*, a spring ephemeral. Botany 96(2): 97-109.

Ebringerova, A., Hromadkova, Z., Heinze, T., 2005. Hemicellulose. *In*: Polysaccharides 1: Structure, Characterization and Use. *Edited by* Heinze, T., Springer-Verlag Berlin Heidelberg, pp. 1-67.

Edlund, E., Novak, O., Karady, M., Ljung, K., Jansson, S., 2017. Contrasting patterns of cytokinins between years in senescing aspen leaves. Plant, Cell & Environment 40(5): 622-634.

Eveland, A.L., Jackson, D.P., 2012. Sugars, signalling, and plant development. Journal of Experimental Botany 63(9): 3367-3377.

Farrar, J.F., 1993. Sink strength: What is it and how do we measure it ? A summary. Plant, Cell & Environment 16(9): 1045-1046.

Farrar, J.F., 1996. Sinks—integral parts of a whole plant. Journal of Experimental Botany 47: 1273-1279.

Feller, U., Fischer, A., 1994. Nitrogen-metabolism in senescing leaves. Critical Reviews in Plant Sciences 13(3): 241-273.

Figueroa, C.M., Lunn, J.E., 2016. A tale of two sugars: trehalose 6-phosphate and sucrose. Plant Physiology 172(1): 7-27.

Fischer, R.A., 2007. Understanding the physiological basis of yield potential in wheat. The Journal of Agricultural Science 145(2): 99-113.

Fischer, R.L., Bennett, A.B., 1991. Role of cell wall hydrolases in fruit ripening. Annual Review of Plant Physiology and Plant Molecular Biology 42(1): 675-703.

Florez-Sarasa, I.D., Bouma, T.J., Medrano, H., Azcon-Bieto, J., Ribas-Carbo, M., 2007. Contribution of the cytochrome and alternative pathways to growth respiration and maintenance respiration in *Arabidopsis thaliana*. Physiologia Plantarum 129(1): 143-151.

Foyer, C., Furbank, R., Harbinson, J., Horton, P., 1990. The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. Photosynthesis Research 25(2): 83-100.

Foyer, C.H., Paul, M.J., 2001. Source–sink relationships. *In*: Encyclopedia of Life Sciences. *Edited by*, Nature Publishing Group, London, pp. 1-11.

Fujita, K., Kai, Y., Takayanagi, M., El-Shemy, H., Adu-Gyamfi, J.J., Mohapatra, P.K., 2004. Genotypic variability of pigeonpea in distribution of photosynthetic carbon at low phosphorus level. Plant Science 166(3): 641-649.

Gan, S.S., Amasino, R.M., 1995. Inhibition of leaf senescence by autoregulated production of cytokinin. Science 270(5244): 1986-1988.

Gan, S.S., Amasino, R.M., 1997. Making sense of senescence - Molecular genetic regulation and manipulation of leaf senescence. Plant Physiology 113(2): 313-319.

Gandin, A., Gutjahr, S., Dizengremel, P., Lapointe, L., 2011. Source-sink imbalance increases with growth temperature in the spring geophyte *Erythronium americanum*. Journal of Experimental Botany 62(10): 3467-3479.

Gandin, A., Lapointe, L., Dizengremel, P., 2009. The alternative respiratory pathway allows sink to cope with changes in carbon availability in the sink-limited plant *Erythronium americanum*. Journal of Experimental Botany 60(15): 4235-4248.

Gechev, T.S., Hille, J., Woerdenbag, H.J., Benina, M., Mehterov, N., Toneva, V., Fernie, A.R., Mueller-Roeber, B., 2014. Natural products from resurrection plants: Potential for medical applications. Biotechnology Advances 32(6): 1091-1101.

Gibson, S.I., 2004. Sugar and phytohormone response pathways: navigating a signalling network. Journal of Experimental Botany 55(395): 253-264.

Gille, S., Cheng, K., Skinner, M.E., Liepman, A.H., Wilkerson, C.G., Pauly, M., 2011. Deep sequencing of voodoo lily (*Amorphophallus konjac*): an approach to identify relevant genes involved in the synthesis of the hemicellulose glucomannan. Planta 234(3): 515-526.

Giraud, E., Ho, L.H., Clifton, R., Carroll, A., Estavillo, G., Tan, Y.-F., Howell, K.A., Ivanova, A., Pogson, B.J., Millar, A.H., 2008. The absence of ALTERNATIVE OXIDASE1a in Arabidopsis results in acute sensitivity to combined light and drought stress. Plant Physiology 147(2): 595-610.

Goldschmidt, E.E., Huber, S.C., 1992. Regulation of photosynthesis by end-product accumulation in leaves of plants storing starch, sucrose, and hexose sugars. Plant Physiology 99(4): 1443-1448.

Gombert, J., Etienne, P., Ourry, A., Le Dily, F., 2006. The expression patterns of SAG12/Cab genes reveal the spatial and temporal progression of leaf senescence in *Brassica napus* L. with sensitivity to the environment. Journal of Experimental Botany 57(9): 1949-1956.

Gomez, L., Jordan, M.O., Adamowicz, S., Leiser, H., Pagès, L., 2003. Du prélèvement au dosage: réflexions sur les problèmes posés par la mesure des glucides non structuraux chez les végétaux ligneux. Cahiers Agricultures 12(6): 369-386.

Gomez, L.D., Steele-King, C.G., Jones, L., Foster, J.M., Vuttipongchaikij, S., McQueen-Mason, S.J., 2009. Arabinan metabolism during seed development and germination in *Arabidopsis*. Molecular Plant 2(5): 966-976.

González-Meler, M.A., Ribas-Carbo, M., Giles, L., Siedow, J.N., 1999. The effect of growth and measurement temperature on the activity of the alternative respiratory pathway. Plant Physiology 120(3): 765-772.

Griffiths, C.A., Paul, M.J., Foyer, C.H., 2016. Metabolite transport and associated sugar signalling systems underpinning source/sink interactions. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1857(10): 1715-1725.

Gutjahr, S., Lapointe, L., 2008. Carbon dioxide enrichment does not reduce leaf longevity or alter accumulation of carbon reserves in the woodland spring ephemeral *Erythronium americanum*. Annals of Botany 102(5): 835-843.

Haberer, G., Kieber, J.J., 2002. Cytokinins. New insights into a classic phytohormone. Plant Physiology 128(2): 354-362.

Hansen, J., Møller, I., 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Analytical Biochemistry 68(1): 87-94.

Hanson, J., Smeekens, S., 2009. Sugar perception and signaling - an update. Current Opinion in Plant Biology 12(5): 562-567.

Hemrika‐Wagner, A.M., Verschoor, E.J., Plas, L.H., 1983. Alternative pathway respiration in vivo of potato tuber callus grown at various temperatures. Physiologia Plantarum 59(3): 369-374.

Hendry, G., 1987. The ecological significance of fructan in a contemporary flora. New Phytologist 106(s1): 201-216.

Hendry, G.A.F., 1993. Evolutionary origins and natural functions of fructans – a climatological, biogeographic and mechanistic appraisal. New Phytologist 123(1): 3-14.

Hendry, G.A.F., Wallace, R.K., 1993. The origin, distribution, and evolutionary significance of fructans. *In*: Science and Technology of Fructans. *Edited by* Suzuki, M., Chatterton, N.J., Boca Raton, FL, USA: CRC Press, pp. 119-139.

Ho, L.C., 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Annual Review of Plant Physiology and Plant Molecular Biology 39(1): 355-378.

Hoch, G., 2007. Cell wall hemicelluloses as mobile carbon stores in non-reproductive plant tissues. Functional Ecology 21(5): 823-834.

Hoch, G., Popp, M., Körner, C., 2002. Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. Oikos 98(3): 361-374.

IPCC, 2018. Global warming of 1.5 ℃. An IPCC Special Report on the impacts of global warming of 1.5 ℃ above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. *Edited by* V. Masson-Delmotte, P. Zhai, H. O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, T. Waterfield. Retrieved from https://www.ipcc.ch/sr15/.

Jackson, S.D., 1999. Multiple signaling pathways control tuber induction in potato. Plant Physiology 119(1): 1-8.

Jajić, I., Sarna, T., Szewczyk, G., Strzałka, K., 2015. Changes in production of reactive oxygen species in illuminated thylakoids isolated during development and senescence of barley. Journal of Plant Physiology 184: 49-56.

Jenner, C., Ugalde, T., Aspinall, D., 1991. The physiology of starch and protein deposition in the endosperm of wheat. Functional Plant Biology 18(3): 211-226.

Jeon, J.-S., Ryoo, N., Hahn, T.-R., Walia, H., Nakamura, Y., 2010. Starch biosynthesis in cereal endosperm. Plant Physiology and Biochemistry 48(6): 383-392.

Jeong, M.L., Jiang, H., Chen, H.S., Tsai, C.J., Harding, S.A., 2004. Metabolic profiling of the sink-to-source transition in developing leaves of quaking aspen. Plant Physiology 136(2): 3364-3375.

Jeong, Y.J., Shang, Y., Kim, B.H., Kim, S.Y., Song, J.H., Lee, J.S., Lee, M.M., Li, J.M., Nam, K.H., 2010. BAK7 displays unequal genetic redundancy with BAK1 in brassinosteroid signaling and early senescence in Arabidopsis. Molecules and Cells 29(3): 259-266.

Jing, H.-C., Sturre, M.J.G., Hille, J., Dijkwel, P.P., 2002. *Arabidopsis onset of leaf death* mutants identify a regulatory pathway controlling leaf senescence. The Plant Journal 32(1): 51-63.

Jolivet, Y., Pireaux, J.C., Dizengremel, P., 1990. Changes in properties of barley leaf mitochondria isolated from sodium chloride treated plants. Plant Physiology 94(2): 641-646.

Keeling, P.L., Myers, A.M., 2010. Biochemistry and genetics of starch synthesis. Annual Review of Food Science and Technology 1: 271-303.

Keleş, Y., Öncel, I., 2002. Response of antioxidative defence system to temperature and water stress combinations in wheat seedlings. Plant Science 163(4): 783-790.

Khan, M., Rozhon, W., Poppenberger, B., 2014. The role of hormones in the aging of plants - a mini-review. Gerontology 60(1): 49-55.

Klockeman, D., Pressey, R., Jen, J., 1991. Characterization of cell wall polysaccharides of jicama (*Pachyrrhizus erosus*) and Chinese water chestnut (*Eleocharis dulicis*). Journal of Food Biochemistry 15(5): 317-329.

Koch, K., 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Plant Biology 7(3): 235-246.

Koch, K.E., Zeng, Y., 2002. Molecular approaches to altered C partitioning: Genes for sucrose metabolism. Journal of the American Society for Horticultural Science 127(4): 474-483.

Kopka, J., Schauer, N., Krueger, S., Birkemeyer, C., Usadel, B., Bergmüller, E., Dörmann, P., Weckwerth, W., Gibon, Y., Stitt, M., 2005. GMD@ CSB. DB: the Golm metabolome database. Bioinformatics 21(8): 1635-1638.

Körner, C., 2003. Carbon limitation in trees. Journal of Ecology 91(1): 4-17.

Krapp, A., Stitt, M., 1995. An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. Planta 195(3): 313-323.

Kruger, N.J., ap Rees, T., 1983. Maltose metabolism by pea chloroplasts. Planta 158(2): 179-184.

Kubacka-Zębalska, M., Kacperska, A., 1999. Low temperature-induced modifications of cell wall content and polysaccharide composition in leaves of winter oilseed rape (*Brassica napus* L. var. *oleifera* L.). Plant Science 148(1): 59-67.

Kudoyarova, G.R., Romanova, A.K., Novichkova, N.S., Vysotskaya, L.B., Akhtyamova, Z., Akhiyarova, G.R., Veselov, S.Y., Ivanov, B.N., 2018. Development of sugar beet leaves: contents of hormones, localization of abscisic acid, and the level of products of photosynthesis. Plant Signaling & Behavior 13(6): 7.

Kumar, R., Mukherjee, S., Ayele, B.T., 2018. Molecular aspects of sucrose transport and its metabolism to starch during seed development in wheat: A comprehensive review. Biotechnology Advances 36(4): 954-967.

Lambers, H., 1982. Cyanide-resistant respiration : a non-phosphorylating electron-transport pathway acting as an energy overflow. Physiologia Plantarum 55(4): 478-485.

Lambers, H., Chapin III, F.S., Pons, T.L., 1998. Plant physiological ecology. Springer-Verlag, New York.

Lambers, H., Van der Dijk, S.J., 1979. Cyanide‐resistant root respiration and tap root formation in two subspecies of *Hypochaeris radicata*. Physiologia Plantarum 45(2): 235-239.

Landhäusser, S.M., Chow, P.S., Dickman, L.T., Furze, M.E., Kuhlman, I., Schmid, S., Wiesenbauer, J., Wild, B., Gleixner, G., Hartmann, H., Hoch, G., McDowell, N.G., Richardson, A.D., Richter, A., Adams, H.D., 2018. Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. Tree Physiology 38(12): 1764-1778.

Lapointe, L., 2001. How phenology influences physiology in deciduous forest spring ephemerals. Physiologia Plantarum 113(2): 151-157.

Lapointe, L., Lerat, S., 2006. Annual growth of the spring ephemeral *Erythronium americanum* as a function of temperature and mycorrhizal status. Canadian Journal of Botany 84(1): 39-48.

Larigauderie, A., Körner, C., 1995. Acclimation of leaf dark respiration to temperature in alpine and lowland plant species. Annals of Botany 76(3): 245-252.

Lastdrager, J., Hanson, J., Smeekens, S., 2014. Sugar signals and the control of plant growth and development. Journal of Experimental Botany 65(3): 799-807.

Le Gall, H., Philippe, F., Domon, J.-M., Gillet, F., Pelloux, J., Rayon, C., 2015. Cell wall metabolism in response to abiotic stress. Plants 4(1): 112-166.

Lebot, V., Prana, M.S., Kreike, N., Van Heck, H., Pardales, J., Okpul, T., Gendua, T., Thongjiem, M., Hue, H., Viet, N., 2004. Characterisation of taro (*Colocasia esculenta* (L.) Schott) genetic resources in Southeast Asia and Oceania. Genetic Resources and Crop Evolution 51(4): 381-392.

León, P., Sheen, J., 2003. Sugar and hormone connections. Trends in Plant Science 8(3): 110-116.

Leonardos, E.D., Savitch, L.V., Huner, N., Öquist, G., Grodzinski, B., 2003. Daily photosynthetic and C - export patterns in winter wheat leaves during cold stress and acclimation. Physiologia Plantarum 117(4): 521-531.

Letham, D.S., Smith, N.G., Willcocks, D.A., 2003. Cytokinin metabolism in *Narcissus* bulbs: chilling promotes acetylation of zeatin riboside. Functional Plant Biology 30(5): 525-532.

Leuzinger, S., Manusch, C., Bugmann, H., Wolf, A., 2013. A sink-limited growth model improves biomass estimation along boreal and alpine tree lines. Global Ecology and Biogeography 22(8): 924-932.

Levigne, S., Ralet, M.-C., Thibault, J.-F., 2002. Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. Carbohydrate Polymers 49(2): 145-153.

Levitt, J., 1972. Responses of plants to environmental stresses. Academic Press, New York.

Lewu, M.N., Adebola, P.O., Afolayan, A.J., 2010. Comparative assessment of the nutritional value of commercially available cocoyam and potato tubers in South Africa. Journal of Food Quality 33(4): 461-476.

Li, B., Xia, J., Wang, Y., Xie, B., 2005. Grain-size effect on the structure and antiobesity activity of konjac flour. Journal of Agricultural and Food Chemistry 53(19): 7404-7407.

Li, L., Zhao, J., Zhao, Y., Lu, X., Zhou, Z., Zhao, C., Xu, G., 2016. Comprehensive investigation of tobacco leaves during natural early senescence via multi-platform metabolomics analyses. Scientific Reports 6(37976): 1-10.

Li, W., Zhang, H., Li, X., Zhang, F., Liu, C., Du, Y., Gao, X., Zhang, Z., Zhang, X., Hou, Z., 2017. Intergrative metabolomic and transcriptomic analyses unveil nutrient remobilization events in leaf senescence of tobacco. Scientific Reports 7(12126): 1-17.

Lim, P.O., Kim, H.J., Nam, H.G., 2007. Leaf senescence. Annual Review of Plant Biology 58: 115-136.

Livingston, D.P., Hincha, D.K., Heyer, A.G., 2009. Fructan and its relationship to abiotic stress tolerance in plants. Cellular and Molecular Life Sciences 66(13): 2007-2023.

Loewus, F.A., 1999. Biosynthesis and metabolism of ascorbic acid in plants and of analogs of ascorbic acid in fungi. Phytochemistry 52(2): 193-210.

Loveys, B.R., Atkinson, L.J., Sherlock, D.J., Roberts, R.L., Fitter, A.H., Atkin, O.K., 2003. Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast - and slow - growing plant species. Global Change Biology 9(6): 895-910.

Lu, Y., Sharkey, T.D., 2006. The importance of maltose in transitory starch breakdown. Plant, Cell & Environment 29(3): 353-366.

Lundmark, M., Hurry, V., Lapointe, L., 2009. Low temperature maximizes growth of *Crocus vernus* (L.) Hill via changes in carbon partitioning and corm development. Journal of Experimental Botany 60(7): 2203-2213.

Lunn, J.E., Delorge, I., Figueroa, C.M., Van Dijck, P., Stitt, M., 2014. Trehalose metabolism in plants. The Plant Journal 79(4): 544-567.

Lunn, J.E., Feil, R., Hendriks, J.H.M., Gibon, Y., Morcuende, R., Osuna, D., Scheible, W.R., Carillo, P., Hajirezaei, M.R., Stitt, M., 2006. Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. Biochemical Journal 397: 139-148.

Mankarios, A.T., Hall, M.A., Jarvis, M.C., Threlfall, D.R., Friend, J., 1980. Cell wall polysaccharides from onions. Phytochemistry 19(8): 1731-1733.

Marcelis, L.F.M., 1996. Sink strength as a determinant of dry matter partitioning in the whole plant. Journal of Experimental Botany 47(Special Issue): 1281-1291.

Martinelli, F., Remorini, D., Saia, S., Massai, R., Tonutti, P., 2013. Metabolic profiling of ripe olive fruit in response to moderate water stress. Scientia Horticulturae 159: 52-58.

Masclaux, C., Valadier, M.-H., Brugière, N., Morot-Gaudry, J.-F., Hirel, B., 2000. Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. Planta 211(4): 510-518.

Matsuo, T., Mizuno, T., 1974. Acetyl groups in native glucomannan from easter lily bulbs. Agricultural and Biological Chemistry 38(2): 465-466.

McDonnell, E., Farrar, J.F., 1993. Respiratory characteristics of isolated barley mitochondria and intact barley roots. Journal of Experimental Botany 44(9): 1485-1490.

McNulty, A.K., Cummins, W.R., 1987. The relationship between respiration and temperature in leaves of the arctic plant *Saxifraga cernua* Plant, Cell & Environment 10(4): 319-325.

Meier, H., Reid, J.S.G., 1982. Reserve polysaccharides other than starch in higher plants. *In*: Plant Carbohydrates I. *Edited by* Loewus, F., Tanner, W., Springer Berlin Heidelberg, pp. 418-471.

Meyer, R.C., Steinfath, M., Lisec, J., Becher, M., Witucka-Wall, H., Törjék, O., Fiehn, O., Eckardt, Ä., Willmitzer, L., Selbig, J., 2007. The metabolic signature related to high plant growth rate in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences 104(11): 4759-4764.

Miller, W.B., 1992. A review of carbohydrate metabolism in geophytes. Acta Horticulturae 325(29): 239-246.

Miller, W.B., Langhans, R.W., 1990. Low-temperature alters carbohydrate-metabolism in Easter lily bulbs. Hortscience 25(4): 463-465.

Møller, I.M., 2001. Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annual Review of Plant Physiology and Plant Molecular Biology 52: 561-591.

Møller, I.M., Bérczi, A., Plas, L.H.W., Lambers, H., 1988. Measurement of the activity and capacity of the alternative pathway in intact plant tissues : identification of problems and possible solutions. Physiologia Plantarum 72(3): 642-649.

Mondal, W.A., Dey, B.B., Choudhuri, M.A., 1985. Proline accumulation as a reliable indicator of monocarpic senescence in rice cultivars. Experientia 41(3): 346-348.

Mondy, N., Mueller, T., 1977. Potato discoloration in relation to anatomy and lipid composition. Journal of Food Science 42(1): 14-18.

Moschen, S., Bengoa Luoni, S., Di Rienzo, J.A., Caro, M.d.P., Tohge, T., Watanabe, M., Hollmann, J., González, S., Rivarola, M., García - García, F., 2016. Integrating transcriptomic and metabolomic analysis to understand natural leaf senescence in sunflower. Plant Biotechnology Journal 14: 719-734.

Mueller-Roeber, B., Balazadeh, S., 2014. Auxin and its role in plant senescence. Journal of Plant Growth Regulation 33(1): 21-33.

Müller, M., Munné-Bosch, S., 2011. Rapid and sensitive hormonal profiling of complex plant samples by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Plant Methods 7: 37-48.

Muller, R.N., 1978. The phenology, growth and ecosystem dynamics of *Erythronium americanum* in the Northern hardwood forest. Ecological Monographs 48(1): 1-20.

Munné-Bosch, S., 2005. The role of α-tocopherol in plant stress tolerance. Journal of Plant Physiology 162(7): 743-748.

Munné-Bosch, S., 2007. Aging in perennials. Critical Reviews in Plant Sciences 26(3): 123-138.

Munné-Bosch, S., Schwarz, K., Alegre, L., 1999. Enhanced formation of α-tocopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. Plant Physiology 121(3): 1047-1052.

Muralikrishna, G., Rao, M., 2007. Cereal non-cellulosic polysaccharides: structure and function relationship - An overview. Critical Reviews in Food Science and Nutrition 47(6): 599-610.

Naor, V., Kigel, J., Ben-Tal, Y., Ziv, M., 2008. Variation in endogenous gibberellins, abscisic acid, and carbohydrate content during the growth cycle of colored *Zantedeschia* spp., a tuberous geophyte. Journal of Plant Growth Regulation 27(3): 211-220.

Neuhaus, H.E., Schulte, N., 1996. Starch degradation in chloroplasts isolated from C_3 or CAM (crassulacean acid metabolism)-induced *Mesembryanthemum crystallinum* L. Biochemical Journal 318(3): 945-953.

Noguchi, K., Terashima, I., 2006. Responses of spinach leaf mitochondria to low N availability. Plant, Cell & Environment 29(4): 710-719.

Noodén, L.D., Guiamét, J.J., John, I., 1997. Senescence mechanisms. Physiologia Plantarum 101(4): 746-753.

Obata-Sasamoto, H., Suzuki, H., 1979. Activities of enzymes relating to starch synthesis and endogenous levels of growth regulators in potato stolon tips during tuberization. Physiologia Plantarum 45(3): 320-324.

Ochoa-Villarreal, M., Aispuro, E., Vargas-Arispuro, I., Martínez-Téllez, M., 2012. Plant cell wall polymers: function, structure and biological activity of their derivatives. *In*: Polymerization. *Edited by* De Souza Gomes, A., InTech, Rijeka, Croatia., pp. 63-86.

Orthen, B., 2001a. Sprouting of the fructan - and starch - storing geophyte *Lachenalia minima*: Effects on carbohydrate and water content within the bulbs. Physiologia Plantarum 113(3): 308-314.

Orthen, B., 2001b. A survey of the polysaccharide reserves in geophytes native to the winter-rainfall region of South Africa. South African Journal of Botany 67(2): 371-375.

Orthen, B., Wehrmeyer, A., 2004. Seasonal dynamics of non-structural carbohydrates in bulbs and shoots of the geophyte *Galanthus nivalis*. Physiologia Plantarum 120(4): 529-536.

Paul, M.J., Foyer, C.H., 2001. Sink regulation of photosynthesis. Journal of Experimental Botany 52(360): 1383-1400.

Paul, M.J., Lawlor, D.W., Driscoll, S.P., 1990. The effect of temperature on photosynthesis and carbon fluxes in sunflower and rape. Journal of Experimental Botany 41(226): 547-555.

Pauly, M., Gille, S., Liu, L., Mansoori, N., de Souza, A., Schultink, A., Xiong, G., 2013. Hemicellulose biosynthesis. Planta 238(4): 627-642.

Pauly, M., Keegstra, K., 2008. Cell‐wall carbohydrates and their modification as a resource for biofuels. The Plant Journal 54(4): 559-568.

Pavel, E.W., Dejong, T.M., 1993. Source-limited and sink-limited growth periods of developing peach fruits indicated by relative growth-rate analysis. Journal of the American Society for Horticultural Science 118(6): 820-824.

Peaucelle, A., Braybrook, S., Höfte, H., 2012. Cell wall mechanics and growth control in plants: the role of pectins revisited. Frontiers in Plant Science 3: Article 121.

Pellny, T.K., Lovegrove, A., Freeman, J., Tosi, P., Love, C.G., Knox, J.P., Shewry, P.R., Mitchell, R.A.C., 2012. Cell walls of developing wheat starchy endosperm: comparison of composition and RNA-Seq transcriptome. Plant Physiology 158(2): 612-627.

Pintó-Marijuan, M., Munné-Bosch, S., 2014. Photo-oxidative stress markers as a measure of abiotic stress-induced leaf senescence: advantages and limitations. Journal of Experimental Botany 65(14): 3845-3857.

Pollock, C.J., 1986. Fructans and the metabolism of sucrose in vascular plants. New Phytologist 104(1): 1-24.

Poorter, H., Villar, R., 1997. The fate of acquired carbon in plants : chemical composition and construction costs. *In*: Plant Resource Allocation. *Edited by* Bazzaz, F.A., Gras, J., Academic Press, San Diego, California., pp. 39-72.

Pracharoenwattana, I., Zhou, W., Keech, O., Francisco, P.B., Udomchalothorn, T., Tschoep, H., Stitt, M., Gibon, Y., Smith, S.M., 2010. Arabidopsis has a cytosolic fumarase required for the massive allocation of photosynthate into fumaric acid and for rapid plant growth on high nitrogen. The Plant Journal 62(5): 785-795.

Prescha, A., Świędrych, A., Biernat, J., Szopa, J., 2001. Increase in lipid content in potato tubers modified by 14-3-3 gene overexpression. Journal of Agricultural and Food Chemistry 49(8): 3638-3643.

Raison, J.K., 1980. Effect of low temperature on respiration. *In*: The Biochemistry of Plants: A Comprehensive Treatise (USA). *Edited by* Davies, D.D., Academic Press, New York, pp. 613-626.

Ranwala, A.P., Miller, W.B., 1998. Gibberellin$_{4+7}$, benzyladenine, and supplemental light improve postharvest leaf and flower quality of cold-stored 'Starazer' hybrid lilies. Journal of the American Society for Horticultural Science 123(4): 563-568.

Ranwala, A.P., Miller, W.B., 2008. Analysis of nonstructural carbohydrates in storage organs of 30 ornamental geophytes by high-performance anion-exchange chromatography with pulsed amperometric detection. New Phytologist 180(2): 421-433.

Raulier, F., Bernier, P., 2000. Predicting the date of leaf emergence for sugar maple across its native range. Canadian Journal of Forest Research 30(9): 1429-1435.

Reid, J.S.G., 1985. Cell wall storage carbohydrates in seeds: biochemistry of the seed "gums" and "hemicelluloses". Advances in Botanical Research 11: 125-155.

Ribas-Carbo, M., Robinson, S.A., Giles, L., 2005. The application of the oxygen-isotope technique to assess respiratory pathway partitioning. *In*: Plant Respiration: From Cell to Ecosystem. *Edited by* Lambers, H., Ribas-Carbo, M., Springer Netherlands, Dordrecht, pp. pp 31-42.

Risser, P.G., Cottam, G., 1968. Carbohydrate cycles in the bulbs of some spring ephemerals Bulletin of the Torrey Botanical Club 95(4): 359-368.

Robakidze, E.A., Bobkova, K.S., 2003. Carbohydrate accumulation in Siberian spruce needles of various ages. Russian Journal of Plant Physiology 50(4): 509-515.

Roessner, U., Wagner, C., Kopka, J., Trethewey, R.N., Willmitzer, L., 2000. Technical advance: simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry. The Plant Journal 23(1): 131-142.

Roitsch, T., Ehneß, R., 2000. Regulation of source/sink relations by cytokinins. Plant Growth Regulation 32: 359-367.

Rolland, F., Baena-Gonzalez, E., Sheen, J., 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. Annual Review of Plant Biology 57: 675-709.

Ruan, Y.-L., 2014. Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annual Review of Plant Biology 65: 33-67.

Ruan, Y.-L., Llewellyn, D.J., Furbank, R.T., 2003. Suppression of sucrose synthase gene expression represses cotton fiber cell initiation, elongation, and seed development. The Plant Cell 15(4): 952-964.

Rubio-Wilhelmi, M.M., Sanchez-Rodriguez, E., Rosales, M.A., Begoña, B., Rios, J.J., Romero, L., Blumwald, E., Ruiz, J.M., 2011. Effect of cytokinins on oxidative stress in tobacco plants under nitrogen deficiency. Environmental and Experimental Botany 72(2): 167-173.

Saha, B.C., 2003. Hemicellulose bioconversion. Journal of Industrial Microbiology and Biotechnology 30(5): 279-291.

Salvador, L.D., Suganuma, T., Kitahara, K., Tanoue, H., Ichiki, M., 2000. Monosaccharide composition of sweetpotato fiber and cell wall polysaccharides from sweetpotato, cassava, and potato analyzed by the high-performance anion exchange chromatography with pulsed amperometric detection method. Journal of Agricultural and Food Chemistry 48(8): 3448-3454.

Sarkar, P., Bosneaga, E., Auer, M., 2009. Plant cell walls throughout evolution: towards a molecular understanding of their design principles. Journal of Experimental Botany 60(13): 3615-3635.

Schädel, C., Blöchl, A., Richter, A., Hoch, G., 2009. Short-term dynamics of nonstructural carbohydrates and hemicelluloses in young branches of temperate forest trees during bud break. Tree Physiology 29(7): 901-911.

Schädel, C., Blöchl, A., Richter, A., Hoch, G., 2010a. Quantification and monosaccharide composition of hemicelluloses from different plant functional types. Plant Physiology and Biochemistry 48(1): 1-8.

Schädel, C., Richter, A., Blöchl, A., Hoch, G., 2010b. Hemicellulose concentration and composition in plant cell walls under extreme carbon source-sink imbalances. Physiologia Plantarum 139(3): 241-255.

Scheller, H.V., Ulvskov, P., 2010. Hemicelluloses. Plant Biology 61(1): 263-289.

Schippers, J.H.M., Jing, H.-C., Hille, J., Dijkwel, P.P., 2007. Developmental and hormonal control of leaf senescence. *In*: Senescence processes in plants. *Edited by* Gan, S., Blackwell Publishing Ltd., Oxford, UK, pp. 145-170.

Schippers, J.H.M., Schmidt, R., Wagstaff, C., Jing, H.-C., 2015. Living to die and dying to live: the survival strategy behind leaf senescence. Plant Physiology 169(2): 914-930.

Schubert, S., Feuerle, R., 1997. Fructan storage in tubers of Jerusalem artichoke: characterization of sink strength. New Phytologist 136(1): 115-122.

Siedow, J.N., Berthold, D.A., 1986. The alternative oxidase: A cyanide-resistant respiratory pathway in higher-plants. Physiologia Plantarum 66(3): 569-573.

Sieger, S.M., Kristensen, B.K., Robson, C.A., Amirsadeghi, S., Eng, E.W.Y., Abdel-Mesih, A., Møller, I.M., Vanlerberghe, G.C., 2005. The role of alternative oxidase in modulating carbon use efficiency and growth during macronutrient stress in tobacco cells. Journal of Experimental Botany 56(416): 1499-1515.

Smeekens, S., Ma, J., Hanson, J., Rolland, F., 2010. Sugar signals and molecular networks controlling plant growth. Current Opinion in Plant Biology 13(3): 273-278.

Solecka, D., Żebrowski, J., Kacperska, A., 2008. Are pectins involved in cold acclimation and de-acclimation of winter oil-seed rape plants? Annals of Botany 101(4): 521-530.

Sørensen, I., Pettolino, F.A., Wilson, S.M., Doblin, M.S., Johansen, B., Bacic, A., Willats, W.G., 2008. Mixed-linkage (1-->3),(1-->4)-β-D-glucan is not unique to the Poales and is an abundant component of *Equisetum arvense* cell walls. The Plant Journal 54(3): 510-521.

Steingröver, E., 1981. The relationship between cyanide-resistant root respiration and the storage of sugars in the taproot in *Daucus carota* L. Journal of Experimental Botany 32(130): 911-919.

Strand, Å., Hurry, V., Gustafsson, P., Gardeström, P., 1997. Development of *Arabidopsis thaliana* leaves at low temperatures releases the suppression of photosynthesis and photosynthetic gene expression despite the accumulation of soluble carbohydrates. The Plant Journal 12(3): 605-614.

Sturm, A., Tang, G.-Q., 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. Trends in Plant Science 4(10): 401-407.

Suttle, J.C., 2004. Physiological regulation of potato tuber dormancy. American Journal of Potato Research 81(4): 253-262.

Taiz, L., Zeiger, E., 2010. Plant physiology, Fifth Edition. Sinauer Associates, Inc., Sunderland, Massachusetts, U.S.A.

Tang, G.-Q., Sturm, A., 1999. Antisense repression of sucrose synthase in carrot (*Daucus carota* L.) affects growth rather than sucrose partitioning. Plant Molecular Biology 41(4): 465-479.

Taylor, R.J., Pearcy, R.W., 1976. Seasonal patterns of the CO_2 exchange characteristics of understory plants from a deciduous forest. Canadian Journal of Botany 54(10): 1094-1103.

Thitisaksakul, M., Jiménez, R.C., Arias, M.C., Beckles, D.M., 2012. Effects of environmental factors on cereal starch biosynthesis and composition. Journal of Cereal Science 56(1): 67-80.

Thomas, H., 2013. Senescence, ageing and death of the whole plant. New Phytologist 197(3): 696-711.

Thomas, M., Thibault, J.-F., 2002. Cell-wall polysaccharides in the fruits of Japanese quince (*Chaenomeles japonica*): extraction and preliminary characterisation. Carbohydrate Polymers 49(3): 345-355.

Turgeon, R., 1989. The sink-source transition in leaves. Annual Review of Plant Biology 40: 119-138.

Turquois, T., Rinaudo, M., Taravel, F.R., Heyraud, A., 1999. Extraction of highly gelling pectic substances from sugar beet pulp and potato pulp: influence of extrinsic parameters on their gelling properties. Food Hydrocolloids 13(3): 255-262.

Valluru, R., Van den Ende, W., 2008. Plant fructans in stress environments: emerging concepts and future prospects. Journal of Experimental Botany 59(11): 2905-2916.

Van de Poel, B., Van Der Straeten, D., 2014. 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: more than just the precursor of ethylene ! Frontiers in Plant Science 5(640): 1-11.

van Doorn, W.G., 2008. Is the onset of senescence in leaf cells of intact plants due to low or high sugar levels ? Journal of Experimental Botany 59(8): 1963-1972.

Van Soest, P.J., 1963. Use of detergents in analysis of fibrous feeds. 2. A rapid method for determination of fiber and lignin. Journal of the Association of Official Agricultural Chemists 46(5): 829-835.

Van Soest, P.J., Wine, R.H., 1967. Use of detergents in analysis of fibrous feeds. 4. Determination of plant cell-wall constituents. Journal of the Association of Official Analytical Chemists 50(1): 50-55.

Vanlerberghe, G.C., 2013. Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. International Journal of Molecular Sciences 14(4): 6805-6847.

Vanlerberghe, G.C., McIntosh, L., 1992. Lower growth temperature increases alternative pathway capacity and alternative oxidase protein in tobacco. Plant Physiology 100(1): 115-119.

Visser, R.G.F., Vreugdenhil, D., Hendriks, T., Jacobsen, E., 1994. Gene expression and carbohydrate content during stolon to tuber transition in potatoes (*Solanum tuberosum*). Physiologia Plantarum 90(2): 285-292.

Vogel, J., 2008. Unique aspects of the grass cell wall. Current Opinion in Plant Biology 11(3): 301-307.

Voronina, T., Seredenin, S., 1988. Analysis of the mechanism of psychotropic action of 3-hydroxypyridine derivative. Annali dell'Istituto Superiore di Sanità 24: 461-466.

Wagner, A.M., Wagner, M.J., Moore, A.L., 1998. *In vivo* ubiquinone reduction levels during thermogenesis in Araceae. Plant Physiology 117(4): 1501-1506.

Wang, C.Y., Cheng, S.H., Kao, C.H., 1982. Senescence of rice leaves. VII. Proline accumulation in senescing excised leaves. Plant Physiology 69(6): 1348-1349.

Wang, J., Rajakulendran, N., Amirsadeghi, S., Vanlerberghe, G.C., 2011. Impact of mitochondrial alternative oxidase expression on the response of *Nicotiana tabacum* to cold temperature. Physiologia Plantarum 142(4): 339-351.

Watanabe, M., Balazadeh, S., Tohge, T., Erban, A., Giavalisco, P., Kopka, J., Mueller-Roeber, B., Fernie, A.R., Hoefgen, R., 2013. Comprehensive dissection of spatiotemporal metabolic shifts in primary, secondary, and lipid metabolism during developmental senescence in Arabidopsis. Plant Physiology 162(3): 1290-1310.

Waters, S.P., Martin, P., Lee, B.T., 1984. The influence of sucrose and abscisic acid on the determination of grain number in wheat. Journal of Experimental Botany 35(6): 829-840.

Weger, H.G., Guy, R.D., 1991. Cytochrome and alternative pathway respiration in white spruce (*Picea glauca*) roots. Effects of growth and measurement temperature. Physiologia Plantarum 83(4): 675-681.

Weiser, R.L., Wallner, S.J., Waddell, J.W., 1990. Cell wall and extensin mRNA changes during cold acclimation of pea seedlings. Plant Physiology 93(3): 1021-1026.

Wen, F., Zhu, Y., Hawes, M.C., 1999. Effect of pectin methylesterase gene expression on pea root development. The Plant Cell 11(6): 1129-1140.

White, A.C., Rogers, A., Rees, M., Osborne, C.P., 2016. How can we make plants grow faster? A source-sink perspective on growth rate. Journal of Experimental Botany 67(1): 31-45.

Wiegand, C.L., Cuellar, J.A., 1981. Duration of grain filling and kernel weight of wheat as affected by temperature. Crop Science 21(1): 95-101.

Wingler, A., Purdy, S., MacLean, J.A., Pourtau, N., 2006. The role of sugars in integrating environmental signals during the regulation of leaf senescence. Journal of Experimental Botany 57(2): 391-399.

Wingler, A., Roitsch, T., 2008. Metabolic regulation of leaf senescence: interactions of sugar signalling with biotic and abiotic stress responses. Plant Biology 10: 50-62.

Wingler, A., von Schaewen, A., Leegood, R.C., Lea, P.J., Quick, W.P., 1998. Regulation of leaf senescence by cytokinin, sugars, and light effects on NADH-dependent hydroxypyruvate reductase. Plant Physiology 116(1): 329-335.

Wolf, S., Greiner, S., 2012. Growth control by cell wall pectins. Protoplasma 249: 169-175.

Wolters, H., Jürgens, G., 2009. Survival of the flexible: hormonal growth control and adaptation in plant development. Nature Reviews Genetics 10(5): 305-317.

Yamada, Y., Umehara, M., 2015. Possible roles of strigolactones during leaf senescence. Plants 4(3): 664.

Yamagishi, M., 1998. Effects of culture temperature on the enlargement, sugar uptake, starch accumulation, and respiration of in vitro bulblets of *Lilium japonicum* Thunb. Scientia Horticulturae 73(4): 239-247.

Yamazaki, H., Nishijima, T., Koshioka, M., Miura, H., 2002. Gibberellins do not act against abscisic acid in the regulation of bulb dormancy of *Allium wakegi* Araki. Plant Growth Regulation 36(3): 223-229.

Yemm, E., Cocking, E., Ricketts, R., 1955. The determination of amino-acids with ninhydrin. Analyst 80(948): 209-214.

Yoon, G.M., Kieber, J.J., 2013. 1-Aminocyclopropane-1-carboxylic acid as a signalling molecule in plants. AoB Plants 5: 6.

Yoshida, S., 2003. Molecular regulation of leaf senescence. Current Opinion in Plant Biology 6(1): 79-84.

Zell, M.B., Fahnenstich, H., Maier, A., Saigo, M., Voznesenskaya, E.V., Edwards, G.E., Andreo, C., Schleifenbaum, F., Zell, C., Drincovich, M.F., Maurino, V.G., 2010. Analysis of Arabidopsis with highly reduced levels of malate and fumarate sheds light on the role of these organic acids as storage carbon molecules. Plant Physiology 152(3): 1251-1262.

Zhou, L., Jang, J.-c., Jones, T.L., Sheen, J., 1998. Glucose and ethylene signal transduction crosstalk revealed by an *Arabidopsis* glucose-insensitive mutant. Proceedings of the National Academy of Sciences 95(17): 10294-10299.

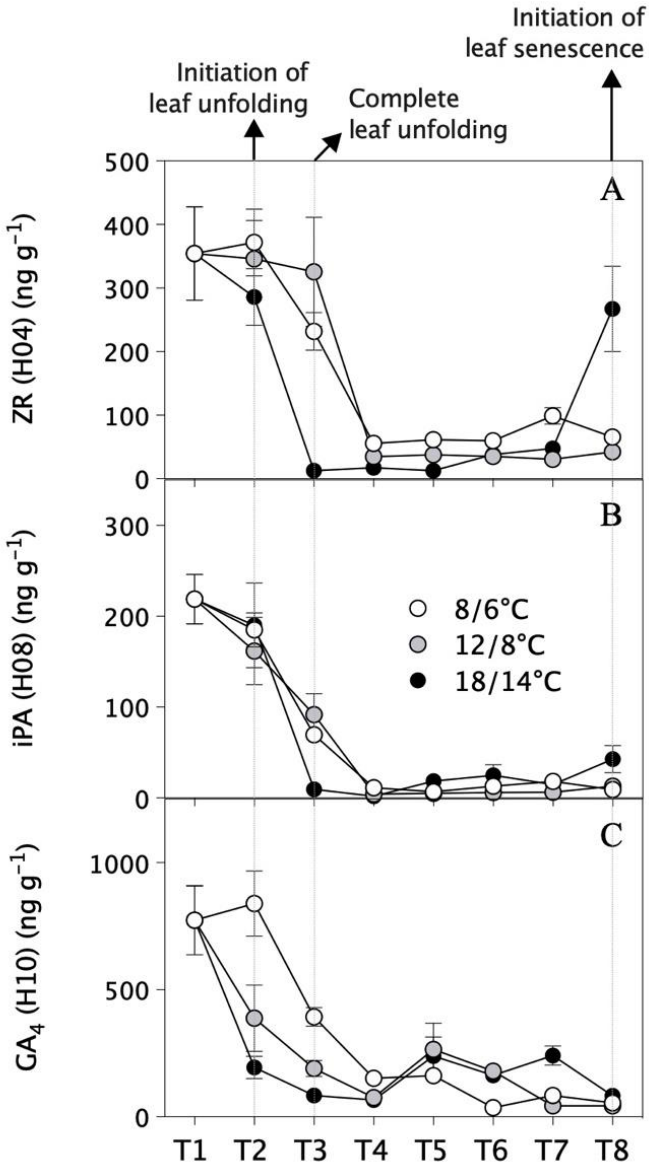
Zimmermann, M.H., 1957. Translocation of organic substances in trees. I. The nature of the sugars in the sieve tube exudate of trees. Plant Physiology 32(4): 288.

Zimmermann, P., Zentgraf, U., 2005. The correlation between oxidative stress and leaf senescence during plant development. Cellular and Molecular Biology Letters 10(3): 515-534.

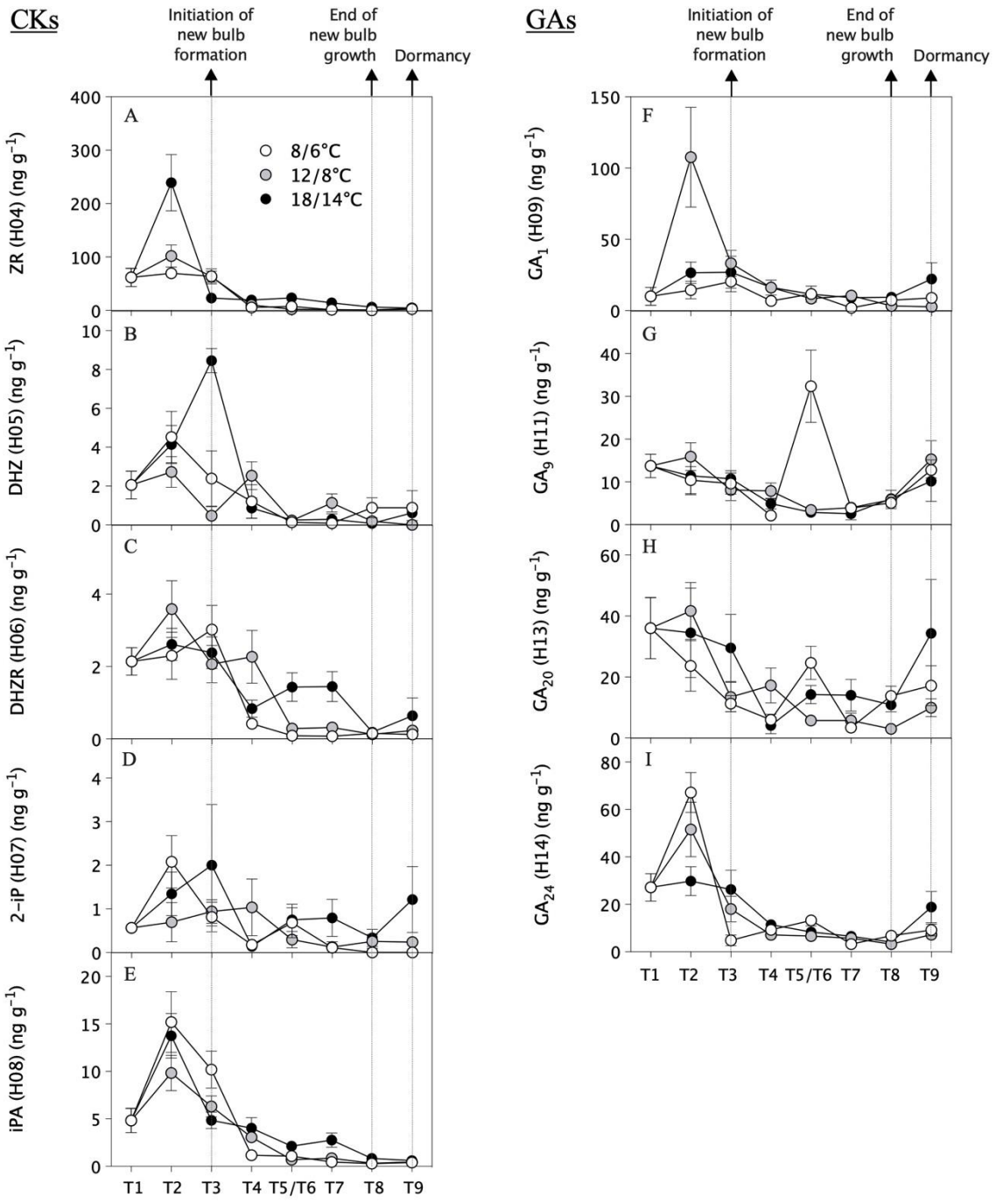
Zrenner, R., Salanoubat, M., Willmitzer, L., Sonnewald, U., 1995. Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). The Plant Journal 7(1): 97-107.

Zwack, P.J., Rashotte, A.M., 2013. Cytokinin inhibition of leaf senescence. Plant Signaling & Behavior 8(7): e24737.

Annexe

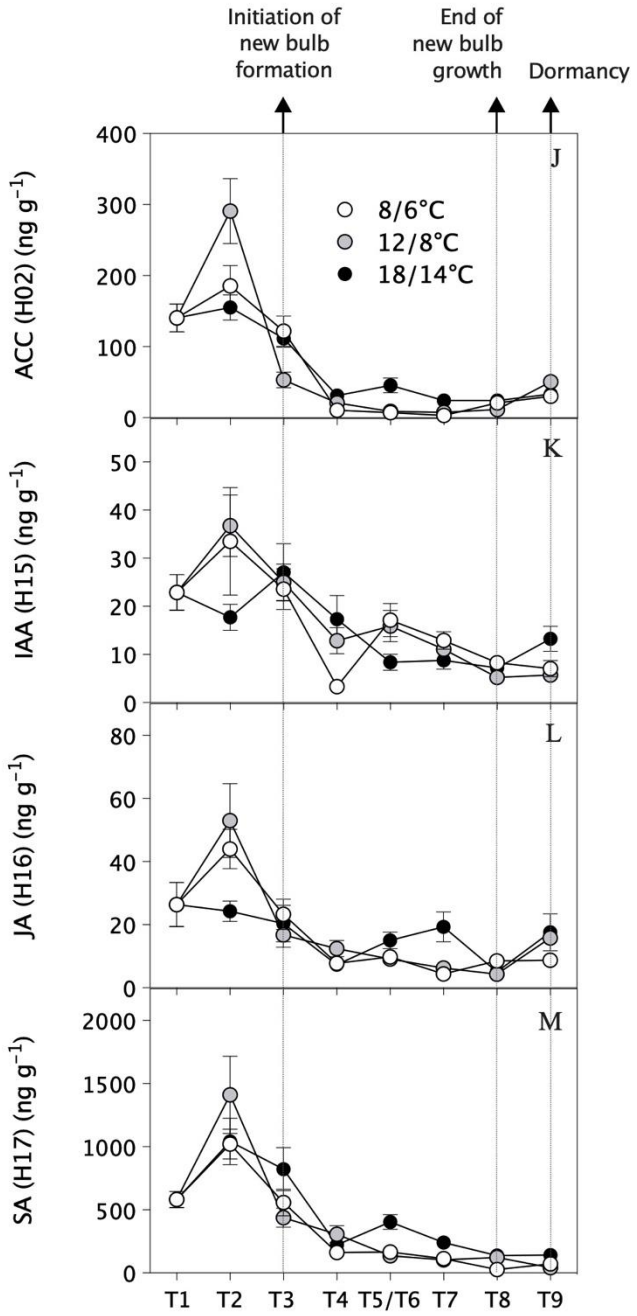


Annex 1. Changes in concentration (ng g^{-1}) of phytohormones over time within leaves of *E. americanum* plants grown under three growth temperature regimes. Only data from phytohormones that were associated with S_L2 [shown in the column entitled "Phenological stage (PCA)" in Table 3-2] are shown (see Table 3-2 for statistical results). Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of leaf unfolding (T2), complete leaf unfolding (T3), and initiation of leaf senescence (T8).

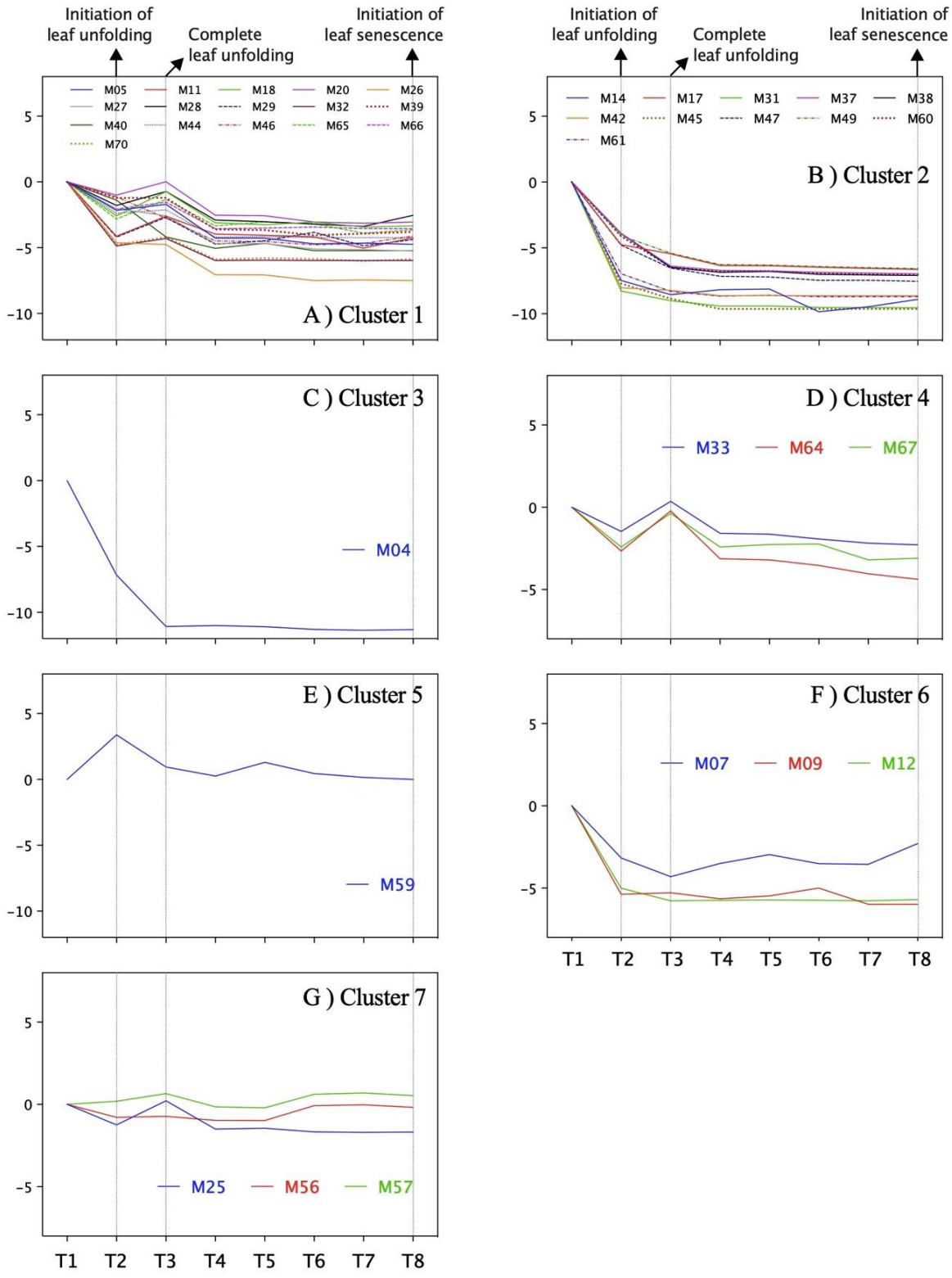


Annex 2. Changes in concentration (ng g^{-1}) of phytohormones over time within bulbs of *E. americanum* plants grown under three growth temperature regimes. Only data from phytohormones that were associated with S_B1 and S_B2 [shown in bold in the column entitled "Phenological stage (PCA)" in Table 3-3] are shown. Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following

phenological stages: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).

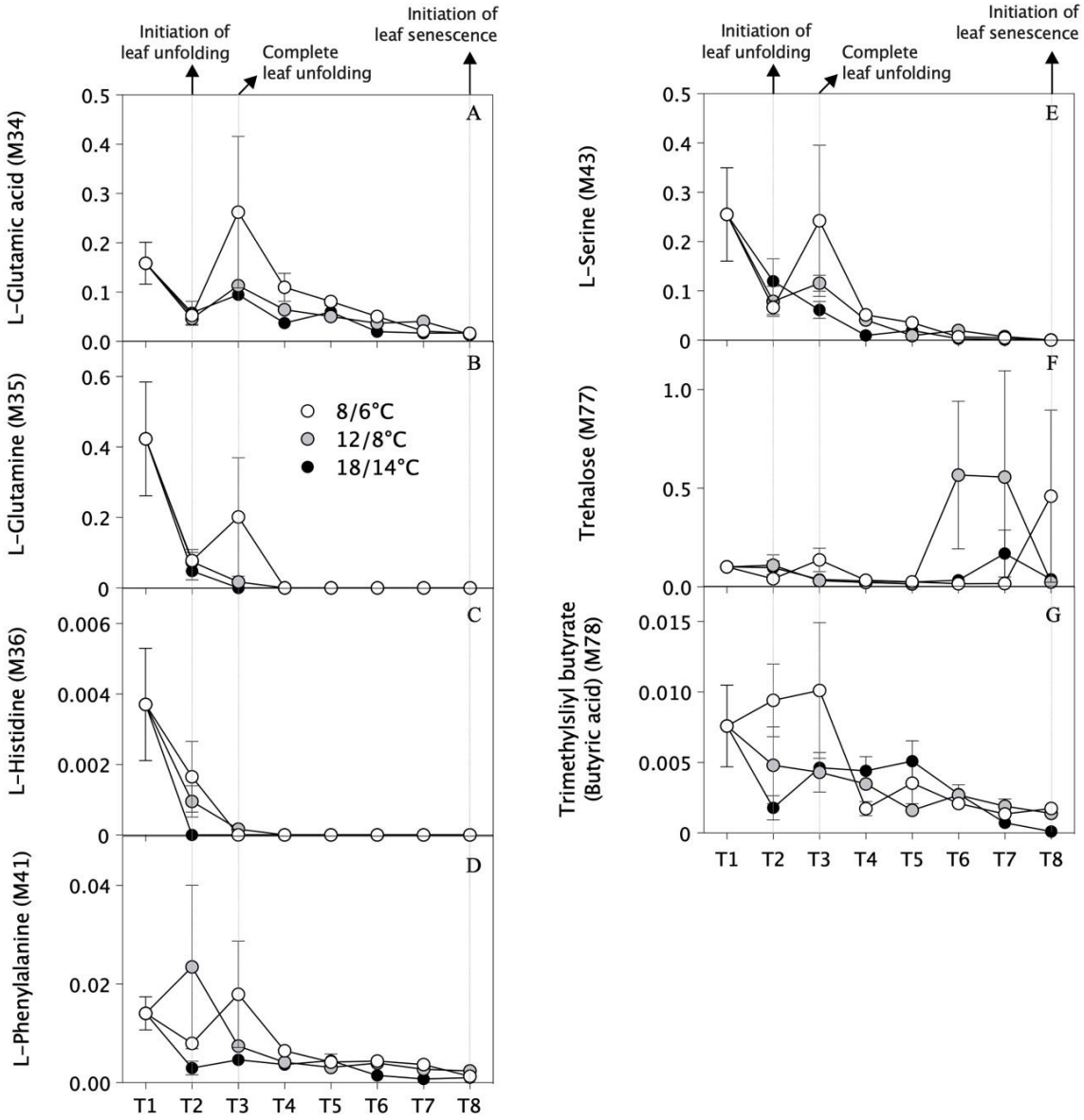


Annex 2. (continued)

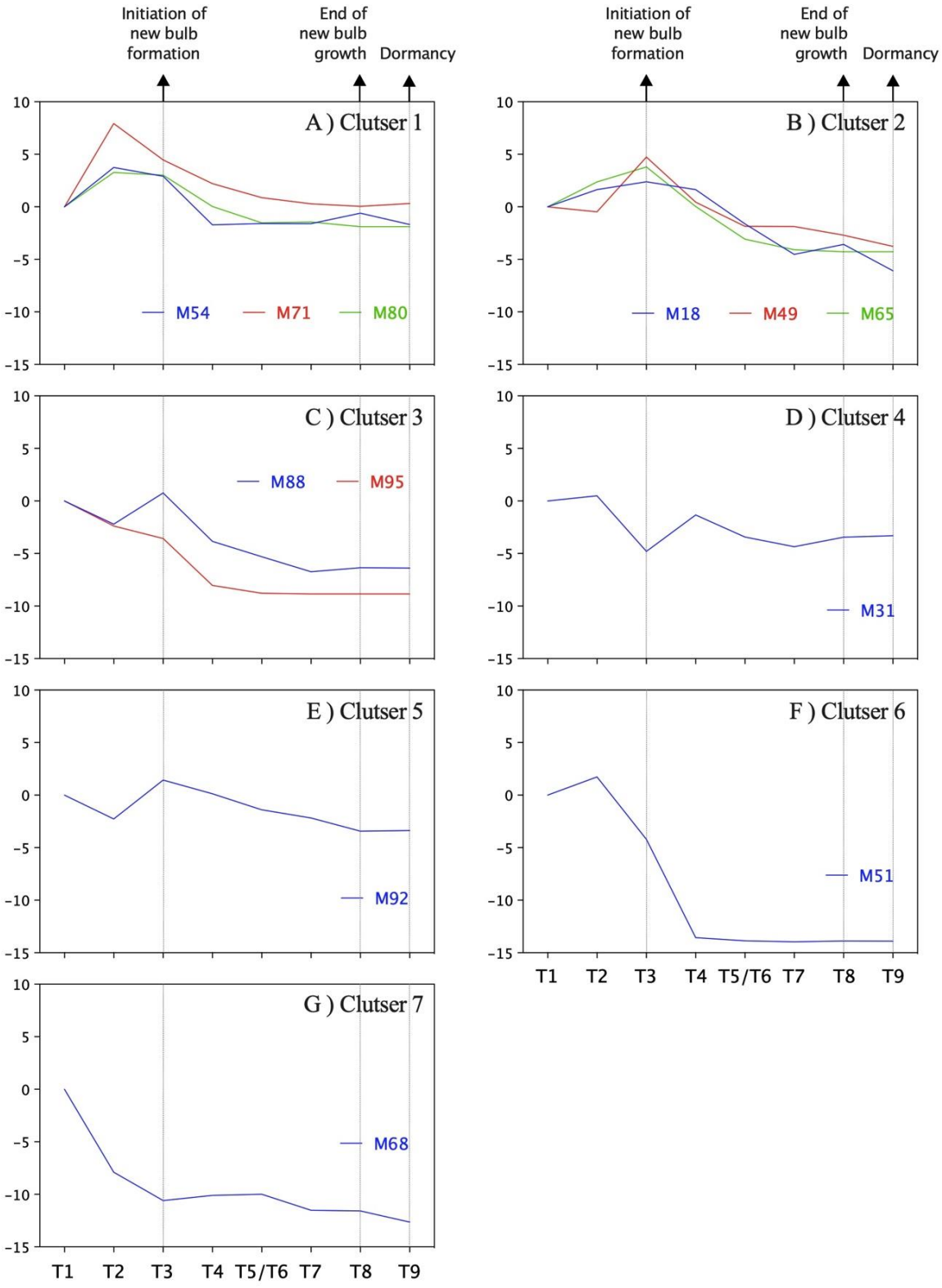


Annex 3. Clusters derived from hierarchical clustering analysis (HCA) showing relative variation in metabolite abundance in leaves of *E. americanum* plants as a function of

harvest time. The clusters were built using data from metabolites that exhibited a simple effect of harvest time (see Table 3-2 for statistical results). Each metabolite profile was zeroed on the mean of T1 and also scaled by dividing each value by the metabolite's average SED (standard error of difference). Only data from metabolites that were associated with S_L2a [shown in the column entitled "Phenological stage (PCA)" in Table 3-2] are shown. Dotted lines indicate the following phenological stages: initiation of leaf unfolding (T2), complete leaf unfolding (T3), and initiation of leaf senescence (T8).

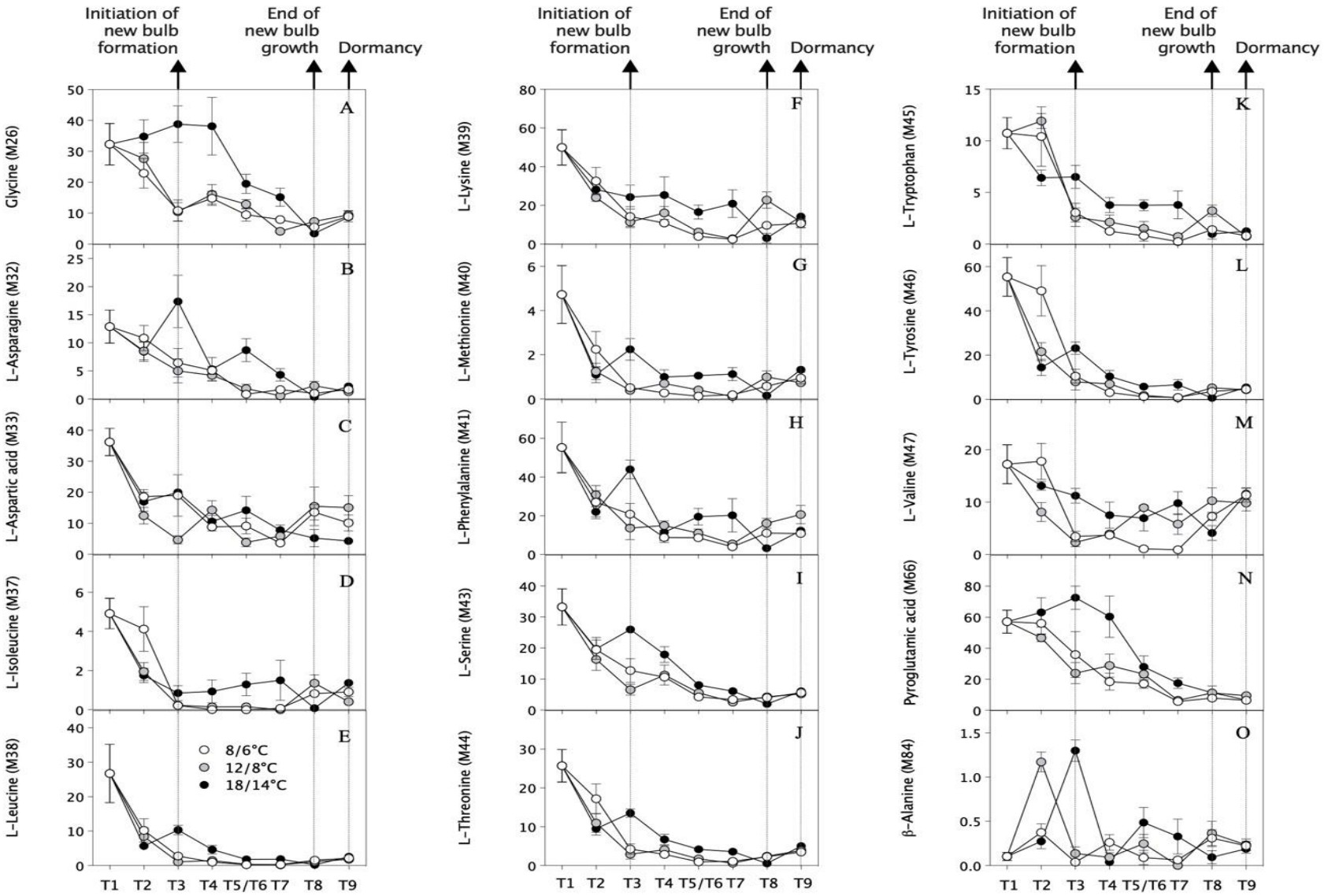


Annex 4. Relative abundance of metabolites in leaves of *E. americanum* plants grown under three growth temperature regimes as a function of harvest time. Only data from metabolites that were associated with S_L2a [shown in the column entitled "Phenological stage (PCA)" in Table 3-2] are shown. Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of leaf unfolding (T2), complete leaf unfolding (T3), and initiation of leaf senescence (T8).

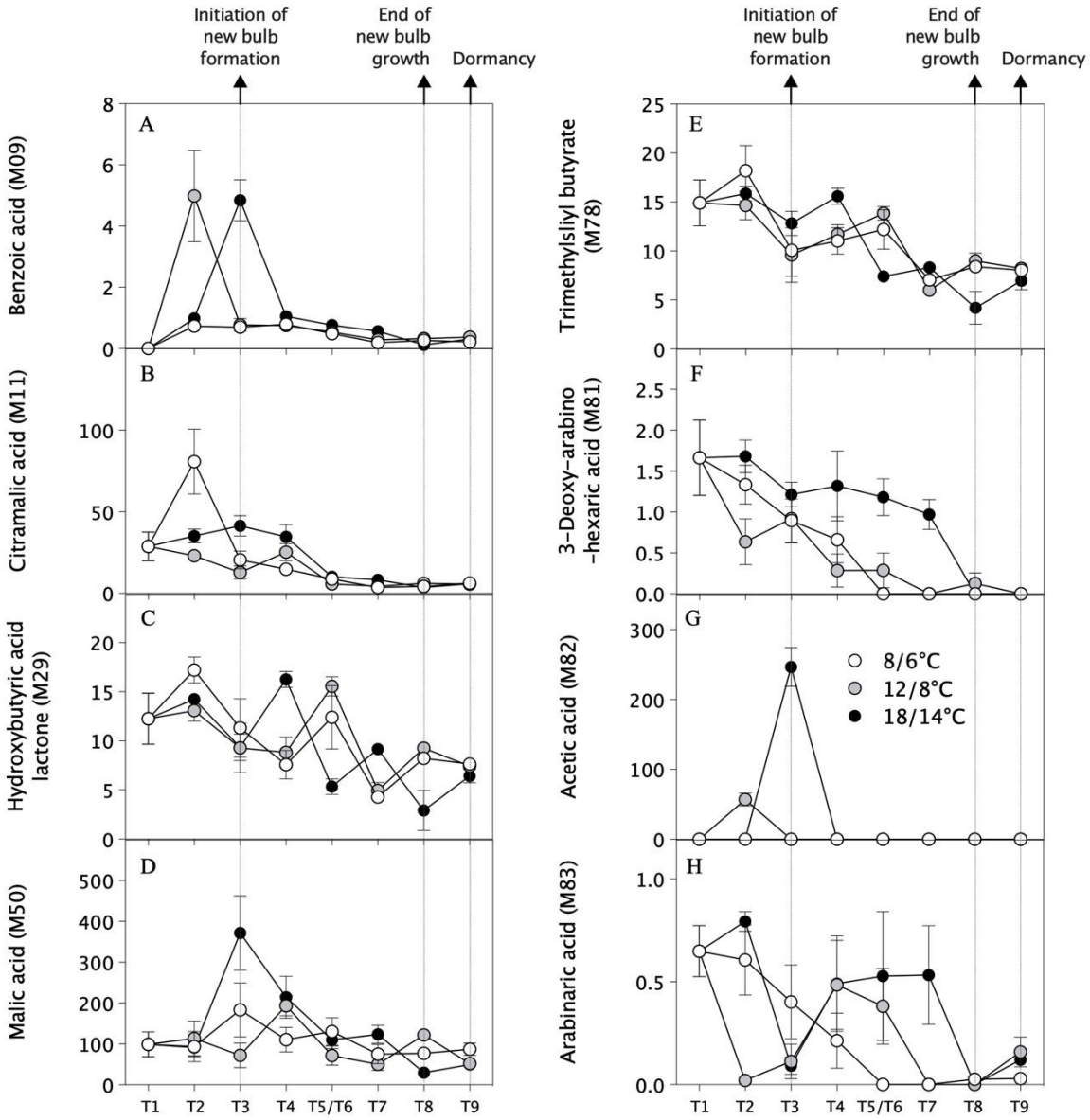


Annex 5. Clusters derived from hierarchical clustering analysis (HCA) showing relative variation in metabolite abundance in bulbs of *E. americanum* plants as a function of harvest

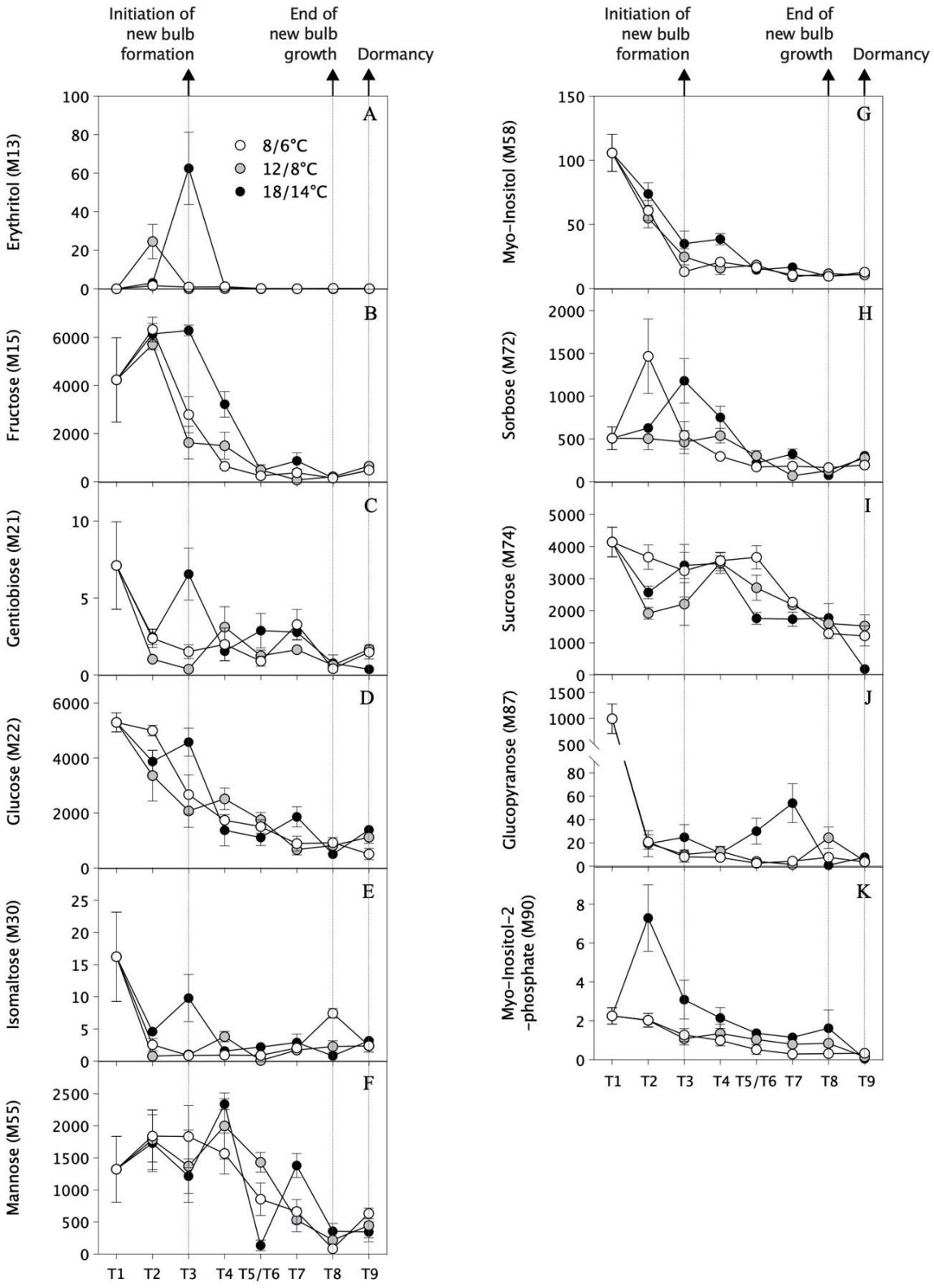
time. The clusters were built using data from metabolites that exhibited a simple effect of harvest time (see Table 3-3 for statistical results). Each metabolite profile was zeroed on the mean of T1 and also scaled by dividing each value by the metabolite's average SED (standard error of difference). Only data from metabolites that were associated with S_B2 [shown in the column entitled "Phenological stage (PCA)" in Table 3-3] are shown. Dotted lines indicate the following phenological stages: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).


Initiation of new bulb formation
End of new bulb growth
Dormancy
Glycine (M26)
L-Asparagine (M32)
L-Aspartic acid (M33)
L-Isoleucine (M37)
L-Leucine (M38)
L-Lysine (M39)
L-Methionine (M40)
L-Phenylalanine (M41)
L-Serine (M43)
L-Threonine (M44)
L-Tryptophan (M45)
L-Tyrosine (M46)
L-Valine (M47)
Pyroglutamic acid (M66)
β-Alanine (M84)
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
8/6°C
12/8°C
18/14°C
T1 T2 T3 T4 T5/T6 T7 T8 T9

Annex 6. Relative abundance of amino acids in bulbs of *E. americanum* plants grown under three growth temperature regimes as a function of harvest time. Only data from metabolites that were associated with S_B2 [shown in the column entitled "Phenological stage (PCA)" in Table 3-3] are shown. Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).

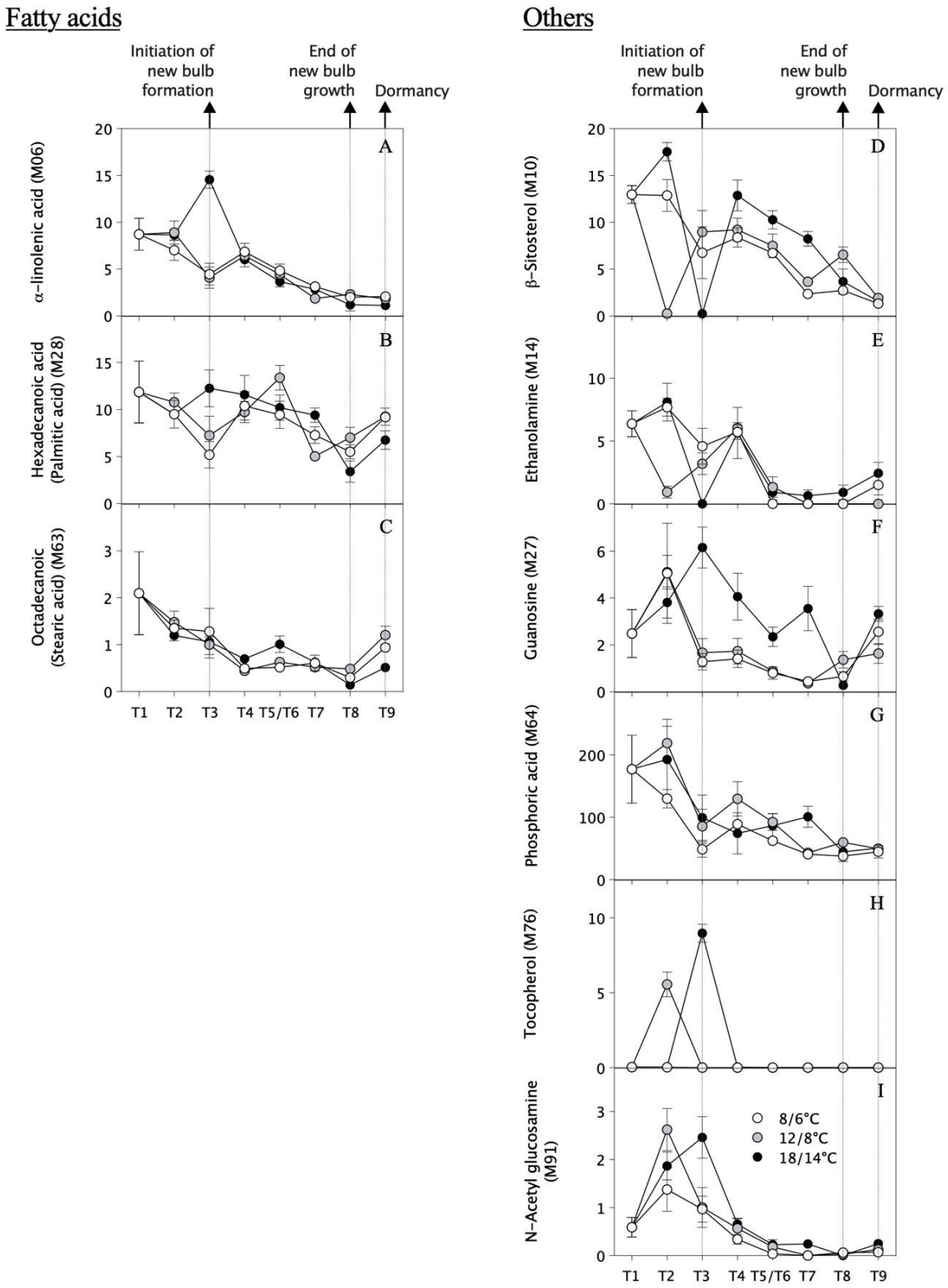


Annex 7. Relative abundance of organic acids in bulbs of *E. americanum* plants grown under three growth temperature regimes as a function of harvest time. Only data from metabolites that were associated with S_B2 [shown in the column entitled "Phenological stage (PCA)" in Table 3-3] are shown. Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).



Annex 8. Relative abundance of sugars and sugar alcohols in bulbs of *E. americanum* plants grown under three growth temperature regimes as a function of harvest time. Only

data from metabolites that were associated with S_B2 [shown in the column entitled "Phenological stage (PCA)" in Table 3-3] are shown. Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).



Annex 9. Relative abundance of fatty acids and other metabolites (class "Others") in bulbs of *E. americanum* plants grown under three growth temperature regimes as a function of

harvest time. Only data from metabolites that were associated with S_B2 [shown in the column entitled “Phenological stage (PCA)” in Table 3-3] are shown. Means ± SEM (standard errors of mean) of five plants are presented. Dotted lines indicate the following phenological stages: initiation of new bulb formation (T3), end of new bulb growth (T8), and dormancy (T9).