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Caractérisation des conditions de culture propices à l'ail des bois en système agroforestier

Mémoire

Pierre-Paul Dion

Maîtrise en biologie
Maître ès sciences (M. Sc.)

Québec, Canada

Résumé

L'ail des bois (*Allium tricoccum*) est une éphémère printanière prisée par les consommateurs. La récolte abusive a mené à la disparition de plusieurs populations au Québec. La culture sous couvert forestier pourrait permettre une exploitation durable de cette espèce vulnérable. Nos travaux ont montré que la plantation sous une voûte forestière à fermeture tardive favorise la croissance de l'ail des bois, qui retarde alors sa sénescence et s'acclimate à une plus grande disponibilité de lumière. Une faible densité de plantation favorise la croissance et la reproduction individuelle, mais se traduit par un rendement plus faible par surface cultivée. La récolte d'une partie des bulbes en populations naturelles denses stimule légèrement la croissance post-récolte. La récolte des feuilles n'a pas d'impact sur la survie, mais l'effectuer tardivement et ne récolter qu'une feuille sur deux favorisent la régénération des plants. Ces résultats permettront d'améliorer le rendement de cultures d'ail des bois.

Abstract

Wild leek (*Allium tricoccum*) is a forest spring ephemeral popular amongst consumers. Overharvesting caused a major decline of its natural populations in southern Quebec, Canada. Forest farming could provide a mean of sustainable exploitation of this vulnerable species. According to our experiments, planting under late bud-bursting tree species improves growth of wild leek, which delays its senescence and can acclimate to the higher light availability. A lower planting density improves individual growth and reproduction, but lowers the yield per cultivated area. Partial harvest of the bulbs in dense populations slightly improves post-harvest growth. Harvesting leaves does not affect survival, but delaying it in the season and harvesting only one leaf out of two favors regeneration. These results will allow optimizing yield in wild leek cultures.

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Avant-propos

Ce mémoire compte quatre chapitres et a été rédigé avec insertion d'articles. Les chapitres 1 et 4 sont respectivement une introduction et une conclusion générales. Les chapitres 2 et 3 seront prochainement soumis pour publication dans des revues scientifiques.

Chapitre 2

Dion, P.-P., Bussières, J. et Lapointe, L. Late canopy closure delays senescence and promotes growth of *Allium tricoccum*, a spring ephemeral.

La mise en place du dispositif expérimental a été menée par Julie Bussières. Le suivi démographique des parcelles au Parc national de la Yamaska a été effectué par les employés du Parc. J'ai mené les prises de données sur le terrain et les analyses en laboratoire pour tous les dispositifs en 2012 et 2013. J'ai analysé tous les résultats et rédigé l'article au complet, sous la supervision de ma directrice de projet, Line Lapointe. Cet article sera prochainement soumis pour publication à *American Journal of Botany*.

Chapitre 3

Dion, P.-P., Bussières, J. et Lapointe, L. Sustainable leaf harvesting and impact of plant density in wild leek cultivation plots and natural stands of Southern Quebec, Canada.

La mise en place des dispositifs expérimentaux a été menée par Julie Bussières. Le suivi du dispositif de densité de plantation a été effectué jusqu'en 2011 par des assistants du laboratoire. J'ai mené les prises de données et les suivis de tous les dispositifs en 2012, 2013 et 2014. J'ai analysé tous les résultats et rédigé l'article au complet, sous la supervision de Line Lapointe. Cet article sera prochainement soumis pour publication à *Agroforestry Systems*.

Chapitre 1. Introduction

1.1 Problématique et historique autour de la conservation de l'ail des bois

L'ail des bois (*Allium tricoccum* Ait.) est une plante forestière comestible très recherchée. Traditionnellement, il était consommé au printemps comme premier légume à apparaitre et ses propriétés nutritives étaient les bienvenues suite à l'hiver sans fruits ni légumes frais (Davis & Greenfield, 2002). Dans les années 1970-80, la récolte commerciale de l'ail des bois a mené à la disparition de plusieurs populations (Couillard, 1995). En 1983, la conserverie des Laurentides ltée était le principal fournisseur pour les épiceries québécoises et mettait en conserve de 3 à 6 millions de bulbes chaque année à elle seule. En comptant le marché artisanal, la vente totale au Québec était évaluée entre 290 000 et 580 000 $ par année (Dagenais, 1985), soit 0,6 à 1,1 M$ en monnaie d'aujourd'hui. La perte d'habitats est aussi une cause du déclin des populations. Depuis 1995, l'ail des bois est protégé par la Loi sur les espèces menacées ou vulnérables : il est interdit de modifier son habitat, sa récolte est limitée à 50 bulbes ou plants par année par personne (ou 200 g de n'importe quelle partie, par exemple les graines) et son commerce est interdit. Malgré la loi, l'ail des bois est encore la cible de braconnage et ses habitats naturels sont toujours en déclin. Mettre en place des cultures d'ail des bois et légaliser le commerce des bulbes issus de ces cultures pourrait diminuer la pression sur les populations naturelles, tout en ouvrant un nouveau marché pour les producteurs et les consommateurs. C'est dans ce contexte que s'inscrit ce projet de recherche, avec pour but d'établir les conditions de culture propices à l'ail des bois en système agroforestier.

1.2 Présentation de l'espèce

L'ail des bois, *Allium tricoccum* Ait., est une plante herbacée printanière pérenne. Ses une à trois feuilles se déploient au printemps suite à la fonte de la neige et, au Québec, atteignent leur pleine ouverture vers la fin avril ou début mai. Elles profitent des conditions de pleine lumière existant en forêt avant le débourrement des bourgeons d'arbres, puis sénescent quelques semaines seulement après le déploiement de leurs feuilles, lorsque le couvert forestier s'est refermé. La hampe florale pousse pendant la sénescence des feuilles et une ombelle de fleurs complètes se déploie en juin et en juillet. Les graines atteignent la

maturité vers la fin août. La reproduction sexuée permet surtout le brassage génétique et la dispersion, car la plus grande part de la reproduction et de la croissance des populations se fait de façon asexuée par la division du bulbe (Jones, 1979; Nault & Gagnon, 1988, 1993). Les réserves de carbone et de nutriments sont accumulées dans le bulbe, qui emmagasine les sucres pendant la période de photosynthèse et récupère la plupart des nutriments des feuilles à leur sénescence (Nault & Gagnon, 1988). À partir de la graine, la croissance d'un plant jusqu'à l'atteinte de la maturité sexuelle peut prendre de 7 à 10 ans (Nantel et al., 1996).

La distribution de l'ail des bois s'étend du Tennessee, É-U, au sud, jusqu'au Manitoba au nord et du Dakota, É-U, à l'ouest jusqu'en Nouvelle-Écosse à l'est (Lavallée, 1978; Dagenais, 1985) (figure 1.1). Au Québec, l'ail des bois est présent de l'extrême sud jusque dans la région de Montmagny, surtout dans l'érablière à caryer et l'érablière à tilleul. Les autres plantes herbacées rencontrées le plus fréquemment dans ces écosystèmes sont la *Viola pensylvanica* Michx., le *Trillium erectum* L. et l'*Erythronium americanum* Ker-Gawl. Toutefois, ces trois espèces sont très répandues, parfois dans des milieux peu propices à l'ail des bois. L'*Asarum canadense* L., le *Trillium grandiflorum* (Michx.) Salisb. et la *Dicentra canadensis* (Goldie) Walp. sont plus rares, mais tout de même fréquemment associés à l'ail des bois, et seraient donc plus à même d'être utilisés comme espèces indicatrices d'un milieu propice à l'ail. Les sols favorables sont les brunisols mélaniques ayant un pH de 6 à 6,6 et un drainage de bon à moyen, quoi qu'une bonne tolérance à l'acidité ait déjà été rapportée par Davis & Greenfield (2002). L'ail est surtout présent à flanc de montagne, dans des pentes de moins de 25° exposées au sud (Lavallée, 1978).

Figure 1.1. Carte de distribution de l'ail des bois. Figure tirée de eFloras (2012).

1.3   Systèmes agroforestiers

Malgré la mise en place de pratiques environnementales en agriculture, les systèmes agricoles basés sur les monocultures ou les simples rotations d'espèces annuelles ne peuvent pas fonctionner à long terme sans apports majeurs de fertilisants et de pesticides. L'intégration de plusieurs espèces sur une même terre permet d'utiliser une plus grande part de sa capacité de production, augmentant ainsi le rendement total (Gordon et al., 2009; Lassoie et al., 2009). Un système agroforestier optimise les services environnementaux de l'agroécosystème, tels que la meilleure fertilité du sol, le maintien ou l'amélioration de la qualité de l'eau, la diminution de l'érosion, le maintien de la biodiversité, le contrôle plus efficace des parasites et des mauvaises herbes, et beaucoup plus (Kohli et al., 2008; Lassoie et al., 2009). Les espèces mises en relation doivent être adéquatement choisies et utiliser différemment les ressources écosystémiques pour que le rendement de la surface cultivée soit effectivement amélioré. Par exemple, les systèmes racinaires des arbres et des herbacées peuvent s'installer à des profondeurs différentes, permettant une utilisation plus optimale de l'eau et des nutriments (Gordon et al., 2009; Bouttier, 2013).

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1.3.1 Culture en boisé

La culture en boisé jumelle la stabilité écologique des forêts naturelles avec la productivité accrue des systèmes agricoles (Chamberlain et al., 2009). Les taux de récolte maximums applicables aux populations naturelles sont souvent très bas, ce qui rend difficile leur exploitation dans un contexte commercial où la rentabilité doit être au rendez-vous. Par exemple, Nantel et al. (1996) ont proposé des taux de récolte annuels maximums pour l'ail des bois (*Allium tricoccum* Ait.) et le ginseng (*Panax quinquefolius* L.) de respectivement 8 et 5 % dans les populations naturelles, en se basant sur la modélisation de la dynamique des populations. L'établissement de parcelles de cultures de plantes forestières est une forme d'agroforesterie particulièrement intéressante pour les propriétaires de boisés. La culture en boisé peut améliorer la productivité par rapport aux populations naturelles et ainsi permettre un taux de récolte annuel plus élevé. En mettant en place de telles cultures, la pression de récolte sur les populations naturelles de ces espèces devrait diminuer, favorisant ainsi leur conservation (De Baets et al., 2007). Plusieurs plantes médicinales sont déjà cultivées sous couvert forestier en Amérique du Nord, telles que le ginseng, l'hydraste du Canada (*Hydrastis canadensis* L.) et la sanguinaire du Canada (*Sanguinaria canadensis* L.). Ce mode de culture semble toutefois marginal et plusieurs espèces sont encore simplement récoltées à l'état sauvage (Lamérant et al., 2008).

En forêt feuillue, la principale ressource pour laquelle les plantes entrent en compétition est la lumière. Les espèces choisies pour ce type de culture doivent donc être bien adaptées aux conditions d'ombre (Gordon et al., 2009). Cependant, l'ail des bois est bien adapté aux milieux boisés, malgré ses besoins élevés en lumière, du fait qu'elle profite de l'abondance de lumière au printemps avant la fermeture de la voûte forestière. Bien que sa culture puisse être possible en milieu ouvert (Vasseur & Gagnon, 1994), sa croissance, sa survie et son taux de germination sont meilleurs en milieu forestier (Davis & Greenfield, 2002). La présence d'adventives en milieu ouvert, pourrait également compromettre sa culture à moyen terme, à moins que des herbicides spécifiques soient disponibles pour réduire la compétition. Il a été montré qu'une culture efficace de l'ail des bois en milieu boisé est possible (Bernatchez et al., 2013). Il reste toutefois à mieux définir les conditions optimales de culture et celles qui favorisent sa rentabilité.

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Le présent projet vise à mieux caractériser certaines des conditions de culture de l'ail des bois en boisé afin d'en optimiser les rendements. Il est divisé en trois volets. Le premier, surnommé « lumière », vise à déterminer l'impact de la composition de la voûte forestière et des conditions de lumière induites par celle-ci sur la croissance de l'ail des bois. Il sera détaillé dans le chapitre 2 de ce mémoire. Le second volet, « coupe de feuilles », permettra de quantifier l'impact de la quantité de feuilles récoltées et du moment de la cueillette sur la croissance des plants d'ail au cours des années subséquentes. Le dernier volet, « densité », consiste en l'étude de l'influence de la densité de plantation de l'ail sur sa croissance, ainsi que de l'influence de la réduction de la densité suite à une récolte dans des populations naturelles sur la croissance des plants restants. Les volets 2 et 3 seront détaillés dans le chapitre 3 du mémoire.

1.4   Présentation théorique du projet

1.4.1   Besoins en lumière

La lumière est un facteur très important dans la croissance des plantes éphémères printanières. Elles profitent de la période de pleine lumière prévalant, en milieu boisé, entre la fonte de la neige et la fermeture de la voûte forestière au printemps pour faire leur photosynthèse et accumuler dans leurs bulbes, cormes, rhizomes ou racines les ressources nécessaires à leur cycle vital annuel (Lapointe, 2001). La longueur de cette période de lumière peut aussi influencer la croissance des plantes printanières : Routhier & Lapointe (2002) ont observé que, pour le *Trillium erectum*, la croissance annuelle est plus forte dans les endroits où le délai entre la fonte de la neige et la fermeture de la voûte forestière est plus long. Ida & Kudo (2008) ont par ailleurs noté une diminution de l'effort reproducteur et de la capacité photosynthétique des feuilles d'une plante de sous-bois, le *Trillium apetalon* Makino, lorsque la lumière est moins abondante au début de la saison de croissance. Cependant, selon Vasseur & Gagnon (1994), la disponibilité de la lumière ne semble pas être un facteur important pour la croissance de l'ail des bois, bien qu'ils reconnaissent qu'une trop faible luminosité peut initier la sénescence, influençant de cette façon la croissance à long terme. Bernatchez & Lapointe (2012) ont d'ailleurs observé que le processus de sénescence de l'ail des bois était beaucoup plus long en chambre de croissance, où la luminosité est constante tout au cours de la saison, qu'en milieu naturel.

La sénescence y a été initiée environ au même moment qu'en milieu naturel, mais a duré beaucoup plus longtemps et la photosynthèse a continué pendant 30 à 50 jours supplémentaires, alors que la durée normale de la sénescence est d'environ 10 jours en milieu naturel. L'ail des bois pourrait donc bénéficier d'une certaine plasticité phénologique permettant de faire varier la durée de sa période d'activité photosynthétique au printemps.

La présence de trouées dans la voûte forestière est un autre facteur pouvant influencer la croissance en modifiant les conditions de lumière, mais aussi l'humidité et la température au sol. Une plus grande quantité de lumière une fois la voûte refermée pourrait retarder la sénescence des plantes printanières (Collins et al., 1985). Toutefois, selon les mêmes auteurs, ce sont surtout les variations de température, accentuées dans les trouées, qui auraient une influence sur la phénologie de ces herbacées. De plus, un printemps plus chaud a le potentiel de raccourcir la période d'activité photosynthétique des feuilles en devançant la sénescence des feuilles, tel que montré chez les éphémères printanières *Erythronium japonicum* Decne. et *Gagea lutea* (L.) Ker-Gawl (Yoshie & Fukuda, 1994; Yoshie, 2008).

La structure de la voûte forestière peut aussi modifier les conditions de lumière par la modulation de l'abondance et de la fréquence des courtes périodes de lumière directe (« *sunflecks* », librement traduit ici par « lumière intermittente »). Cette lumière intermittente peut influencer la photosynthèse des plantes de sous-bois. Une courte période de lumière directe de quelques secondes peut entrainer une fixation de carbone supérieure à la quantité prédite sous éclairage constant d'intensité similaire (Chazdon & Pearcy, 1986). Pendant l'été, en forêt tempérée, environ 50% de la lumière disponible provient de ces courtes périodes de lumière intermittente (Chazdon, 1988). Il a été montré qu'une durée cumulée élevée de lumière intermittente favorise la croissance de l'herbacée de sous-bois *Panax quinquefolius* L. (Fournier et al., 2004). Une modélisation approfondie de l'apport de la lumière intermittente dans les conditions lumineuses printanières en forêt décidue tempérée permettrait une meilleure compréhension des changements environnementaux s'opérant au cours du déploiement des feuilles d'arbres. Toutefois, pendant la période d'activité photosynthétique de l'ail des bois, avant la fermeture de la voûte forestière, une

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très grande part de la lumière disponible est sous forme de lumière directe (Brantley & Young, 2009), et l'efficacité d'utilisation de la lumière intermittente est réduite lorsque les périodes de pleine lumière sont prolongées (Chazdon & Pearcy, 1986). Ceci devrait minimiser l'importance de la lumière intermittente pour la croissance des éphémères printanières. Nous avons donc décidé de ne pas considérer la lumière intermittente dans le présent mémoire afin de focaliser l'analyse sur la croissance de l'ail des bois plutôt que sur la modélisation de l'environnement lumineux. Il serait toutefois très intéressant d'approfondir cet aspect dans le cadre de travaux futurs.

La mesure de la quantité totale de lumière disponible et des relevés de la phénologie des arbres et de l'ail des bois seront au cœur de ce volet du projet. Plusieurs autres variables environnementales devront toutefois être considérées. Par exemple, la présence d'une litière abondante pourrait aider à stabiliser la température ou à conserver l'humidité du sol pendant l'été et ainsi favoriser la croissance et la survie du bulbe (Koorem et al., 2011). Un effet négatif de la litière a cependant été observé sur la germination des graines (Sydes & Grime, 1981; Koorem et al., 2011). Une forte densité de mauvaises herbes pourrait aussi occasionner de la compétition à l'ail des bois, tant pour la lumière qu'au niveau racinaire, affectant négativement sa croissance.

1.4.1.1 *Mesure des conditions lumineuses*

Plusieurs méthodes permettent de mesurer ou d'estimer la radiation active pour la photosynthèse (PAR) qui se rend jusqu'au sol (Parent & Messier, 1996; Comeau et al., 1998; Gendron et al., 1998; Machado & Reich, 1999; Paquette et al., 2007). Les principales sont décrites dans l'annexe 1. La mesure standard directe, lorsque bien appliquée, donne une mesure réelle de la PAR. Toutefois, cette méthode nécessite la mise en place d'au moins un, idéalement deux capteurs de lumière par parcelle, en plus d'installer un enregistreur à proximité. Étant donné le grand nombre de parcelles mises en place sur différents sites pour notre expérience, cela aurait entraîné un coût très élevé. De plus, ces capteurs peuvent facilement être obstrués par une feuille, ce qui causerait la perte de données précieuses. Nous avons donc choisi la méthode des photos hémisphériques. Nous considérons cette méthode mieux adaptée aux besoins de ce projet que les autres méthodes. La diversité des données que l'on peut modéliser à partir des photos et le fait qu'elles

considèrent la position des trouées et la trajectoire du soleil permettent une estimation plus précise de la quantité de lumière atteignant l'ail au cours d'une journée. La prise de photos se fait relativement rapidement sur le terrain, ce qui permet de visiter toutes les parcelles dans un délai raisonnable et ainsi prendre des mesures hebdomadaires dans chacune des parcelles. Afin de contrebalancer la subjectivité de l'analyse, toutes les photos ont été analysées par la même personne.

1.4.1.2 Réponse de la plante à l'irradiance

La densité du couvert forestier est souvent liée au pourcentage de recouvrement du sol par la litière. Effectivement, plus le feuillage et la forêt en générale sont denses, plus la quantité de feuilles formant la litière est élevée (Lebret et al., 2001). Cela crée un problème lors de l'analyse des données : alors que la lumière et le pourcentage de recouvrement par la litière sont statistiquement liés, comment établir avec certitude qu'il y a une relation de causalité entre la lumière et la croissance de l'ail des bois? L'acclimatation des feuilles d'ail elles-mêmes aux conditions de lumière peut nous permettre de confirmer l'effet de la lumière. L'acclimatation à l'ombre induit une baisse de la capacité photosynthétique (Nobel, 1991). Cependant, les mesures d'échanges gazeux prennent beaucoup de temps sur le terrain, ce qui constitue une contrainte importante lorsque la saison de croissance ne dure que 4 à 6 semaines. Deux autres mesures permettant d'évaluer l'acclimatation aux conditions de lumière sont couramment utilisées dans la littérature : le rapport « chlorophylle a : b » (chl a/b) et le rapport des isotopes stables du carbone « $^{13}C : ^{12}C$ » ($\delta^{13}C$).

Une hausse du rapport chl a/b est normalement le résultat d'une acclimatation à des conditions de lumière abondante (Dale & Causton, 1992). Cette hausse est due à une augmentation d'un autre rapport : celui du nombre de photosystèmes I par rapport aux photosystèmes II (PS I/II). Les chloroplastes développés en pleine lumière ont des thylakoïdes moins abondants et contiennent moins de grana que les chloroplastes acclimatés à l'ombre. Les PS I sont positionnés uniquement sur les faces externes des grana et sur les lamelles libres de thylakoïdes. Il y a donc plus de PS I par rapport aux PS II dans les chloroplastes acclimatés à une forte lumière (petits grana et moins abondants) que dans ceux acclimatés à l'ombre (gros grana et plus abondants). Comme le rapport chl a/b est plus élevé dans les PS I que dans les PS II, une augmentation du rapport PS I/II se traduit par

une augmentation du rapport chl a/b (Boardman, 1977; Nobel, 1991). De plus, la taille du complexe antennaire associé au PS II est réduite chez les plantes acclimatées à la forte lumière (Ballottari et al., 2007). Le centre réactionnel des PS ne contient que de la chl a, alors que le complexe antennaire contient les deux types de chlorophylle. La réduction de la taille du complexe antennaire à forte lumière cause donc une augmentation du rapport chl a/b. Cette variation dans le rapport chl a/b peut s'observer d'une plante à l'autre, d'une feuille à l'autre sur un même plant et même parfois d'une cellule à l'autre d'une même feuille (Boardman, 1977; Nobel, 1991).

Une hausse du rapport $^{13}C : ^{12}C$ est normalement le résultat de l'acclimatation de la feuille à une baisse du rapport entre la concentration de carbone intercellulaire et atmosphérique ($C_i : C_a$). Le rapport $^{13}C : ^{12}C$ est habituellement plus faible dans les tissus végétaux que dans l'atmosphère, car la RUBISCO discrimine en faveur du ^{12}C. Une deuxième raison, moins importante, est que le ^{13}C diffuse plus lentement dans la feuille que le ^{12}C (Bowling et al., 2008). Lorsque le taux de photosynthèse est plus élevé, par exemple à forte irradiance, le rapport $C_i : C_a$ tend à diminuer (Ehleringer et al., 1986). La diminution de la disponibilité du CO_2 pour la RUBISCO fait en sorte qu'elle devient moins discriminante par rapport à l'isotope du carbone qu'elle fixe. Il s'ensuit une hausse du rapport $^{13}C : ^{12}C$ (Dawson et al., 2002).

Aux fins d'analyse, le rapport $^{13}C : ^{12}C$ est le plus souvent transformé en $\delta^{13}C$. Le $\delta^{13}C$ est une mesure de la déviation du rapport $^{13}C : ^{12}C$ dans un tissu végétal étudié par rapport à une valeur de référence internationale, dans notre cas le V-PDB (Dawson et al., 2002). Le $\delta^{13}C$ est exprimé en ‰ et prend une valeur négative, car la RUBISCO discrimine toujours en faveur du ^{12}C. Une augmentation du $\delta^{13}C$ (rapprochement de zéro) peut donc être liée à une acclimatation à une forte irradiance. La discrimination en faveur du ^{12}C liée à l'activité enzymatique est en moyenne de l'ordre de -27 ‰ chez les plantes C_3. La diffusion plus rapide du ^{12}C de l'atmosphère aux chloroplastes cause quant à elle une discrimination d'environ -4 ‰ (Farquhar et al., 1982).

Un des facteurs qui influencent le $\delta^{13}C$, soit le Ci est non seulement influencé par le taux de photosynthèse mais également par la conductance stomatique. Comme cette dernière est influencée par l'humidité relative de l'air et la disponibilité de l'eau dans le

sol, la variation du $\delta^{13}C$ est donc le reflet de la variation de l'humidité relative de l'air, de la disponibilité de l'eau dans le sol et de la capacité photosynthétique (Scheidegger et al., 2000). Joindre une mesure du rapport chl a/b à celle du $\delta^{13}C$ est donc nécessaire pour confirmer que les changements de $\delta^{13}C$ reflètent une acclimatation aux conditions de lumière plutôt qu'à la disponibilité en eau.

L'objectif de ce volet du projet sera de déterminer l'influence de la composition du couvert forestier sur la croissance de l'ail des bois, en considérant particulièrement les conditions de lumière induite par ce couvert forestier tout au long de la saison de croissance épigée de l'ail des bois.

1.4.1.3 *Hypothèses et prédictions*

A- Une plus grande disponibilité de la lumière au printemps devrait favoriser la croissance de l'ail des bois en lui permettant de fixer plus de carbone.

Deux paramètres pourraient influencer la disponibilité de lumière. D'abord, une espèce arborescente dont le feuillage forme une voûte forestière moins dense et laisse passer plus de lumière pourrait améliorer suffisamment les conditions d'irradiance au sol pour stimuler la croissance de l'ail. De même, une forêt plus clairsemée ou la présence de trouées dans la voûte forestière pourrait avoir le même effet. Nous prédisons que l'ail des bois aura une meilleure croissance dans une plantation d'arbres et dans les trouées qu'en forêt naturelle (figure 1.2). Elle présentera également une plus grande acclimatation à la pleine lumière dans les plantations et les trouées.

B- L'augmentation de la durée de disponibilité maximale de la lumière avant la fermeture de la voûte forestière devrait favoriser la croissance de l'ail des bois, en retardant la sénescence de ses feuilles.

Une essence d'arbre dont les feuilles débourrent tardivement allonge la période de pleine lumière au sol au printemps. Cela créerait des conditions favorables à l'ail des bois, dont la phénologie s'ajusterait en retardant la sénescence des feuilles, allongeant ainsi sa période photosynthétique et menant à une croissance améliorée (figure 1.2). Nous prédisons donc que la croissance de l'ail des bois sera plus élevée sous une voûte composée de frêne

(*Fraxinus* sp.) ou de chêne rouge (*Quercus rubra* L.) que sous une voûte composée d'érables à sucre (*Acer saccharum* Marshall).
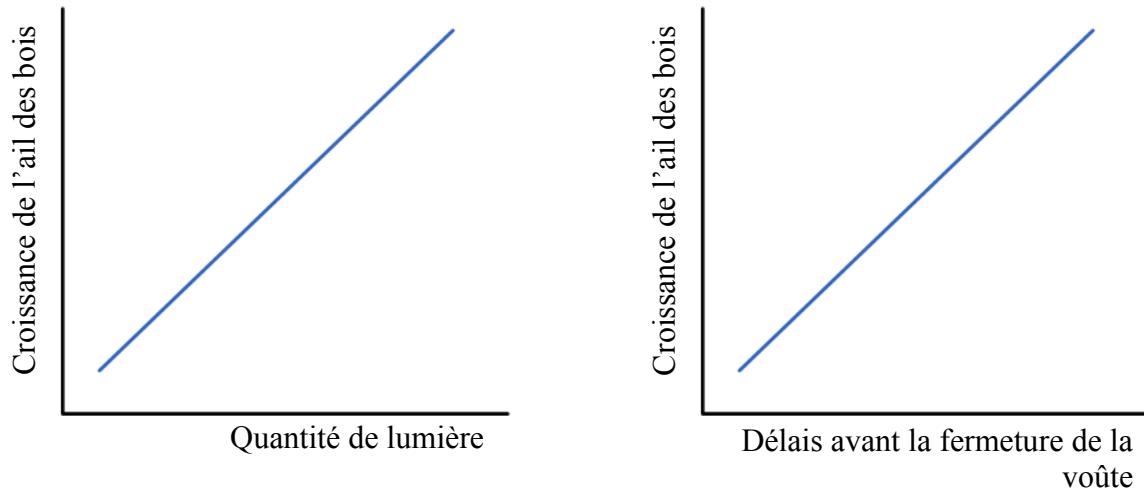
Figure 1.2 Prévision de la variation de la croissance de l'ail des bois selon la quantité de lumière et la date de fermeture de la voûte forestière.

1.4.2   Récolte de feuilles

C'est principalement le bulbe de l'ail des bois qui est cueilli pour la consommation. Les feuilles sont pourtant elles aussi comestibles et leur récolte ne conduit généralement pas à la mort du plant, ce qui pourrait fournir une option plus durable que la récolte du bulbe tout en permettant de profiter des atouts culinaires de cette espèce.

Les feuilles sont toutefois un investissement majeur en termes de biomasse et de nutriments pour l'ail des bois. Après leur plein déploiement, elles comptent pour environ 50% de sa biomasse totale et contiennent plus de 60% de ses principaux nutriments (P, K, Mg), et même jusqu'à 90% de l'azote (N) et du calcium (Ca) (Nault & Gagnon, 1988). L'ail des bois est très efficace dans la récupération de ces nutriments pendant la sénescence des feuilles. La biomasse et la concentration en nutriments maximales de son bulbe sont d'ailleurs atteintes après le début de la sénescence des feuilles. De plus, la photosynthèse demeure active, quoique légèrement diminuée, dans la feuille même une fois la sénescence initiée (Bernatchez & Lapointe, 2012). Même le Ca, normalement immobile dans la plante, semble être récupéré en partie par les racines dans le sol suite à la décomposition de la

feuille (Nault & Gagnon, 1988). La cueillette des feuilles pourrait donc priver le bulbe d'une importante quantité de nutriments et d'énergie.

L'effet de la récolte d'une demi-feuille peut être très différent d'une simple réduction de la fixation de carbone proportionnelle à la surface foliaire perdue. Morrison & Reekie (1995) ont observé une hausse du taux de photosynthèse par unité de surface dans la partie restante d'une feuille partiellement coupée, alors que Zangerl et al. (2002) ont observé le contraire et Nowak & Caldwell (1984) n'ont observé aucun effet. Cette différence dans les résultats serait probablement due à la forme de défoliation : une coupe franche aurait un impact moindre qu'une défoliation aléatoire grâce à une plus courte longueur de coupe (Morrison & Reekie, 1995). Lorsque les feuilles d'une plante sont broutées ou endommagées, il peut aussi se produire un effet de croissance compensatoire. La croissance du plant est alors stimulée de sorte que la croissance subséquente n'est pas directement proportionnelle à la proportion de la plante qui a été endommagée. La croissance compensatoire peut être le résultat de différents mécanismes tels que la hausse de capacité photosynthétique du feuillage restant, la stimulation de la division cellulaire ou la disponibilité de lumière accrue suite à la disparition de feuillage (McNaughton, 1983).

L'impact de la récolte des feuilles sur la croissance n'a pas été étudié directement chez l'ail des bois, mais plusieurs études font état de l'effet de la défoliation chez d'autres *Alliums* et liliacées. Les travaux de Muro et al. (1998); Muro et al. (2000) ont montré l'impact négatif de la défoliation sur le rendement de l'oignon (*Allium cepa*) et de l'ail commercial (*Allium sativum*). Lapointe et al. (2010) ont observé que la défoliation totale effectuée tôt en saison ralentit la croissance de plusieurs liliacées québécoises vivant dans des environnements similaires à celui de l'ail des bois. La survie, la floraison, la surface foliaire, la biomasse, les réserves de carbone, les nutriments et le rapport C/N sont affectés par la défoliation chez le *Trillium erectum*, la *Clintonia borealis* (Raf.) Ait. et le *Maianthemum canadense* Desf., particulièrement lorsque cette défoliation a lieu deux années consécutives. Whigham & Chapa (1999) ont observé que la croissance du corme de l'éphémère printanière *Claytonia virginica* L. est ralentie lorsque la défoliation a lieu tôt en saison. Son effort reproducteur n'est toutefois pas affecté et l'intensité de cette défoliation semble avoir peu d'effet sur la croissance du corme.

L'asperge (*Asparagus officinalis* L.) est une des seules espèces vivaces tempérées dont la récolte peut se comparer à celle des feuilles d'ail des bois. Les racines et le rhizome de l'asperge, une liliacée, sont les seules sources d'énergie du plant pendant la période de récolte au printemps. Les parties comestibles sont les jeunes pousses, les *turions*. Toutefois, contrairement à l'ail des bois, l'asperge a la capacité de produire plusieurs turions, et les producteurs en laissent pousser quelques-uns une fois la récolte des turions terminée, de façon à ce que le plant puisse refaire ses réserves de biomasse pendant la saison de croissance. Le rhizome de l'asperge perd deux fois plus de biomasse pendant la récolte que le total de la biomasse récoltée, en plus de perdre beaucoup de K, P et Na. Le rendement de la récolte suivante est fortement dépendant de l'accumulation de réserves pendant l'été précédent (Adam & Stengel, 1999). La vigueur du plant au cours de l'été précédent est habituellement un bon signe de l'intensité de récolte applicable pour la saison présente (Bergeron et al., 2003).

L'étape phénologique à laquelle a lieu la défoliation influence également son impact sur la croissance. Chez l'ail cultivé, le début de la croissance du bulbe, au moment où les dernières feuilles vertes apparaissent et les premières feuilles commencent leur sénescence est l'étape phénologique où la défoliation a le plus grand impact sur le rendement. Cet impact sur le rendement augmente avec la sévérité de la défoliation. Des conclusions similaires ont été obtenues chez le poireau (*Allium ampeloprasum* L. ssp. *porrum*), où l'impact maximal de la défoliation a été obtenu lorsque la presque totalité des feuilles avaient complété leur développement et que la croissance en diamètre de la tige était bien entamée, à environ 30% du diamètre final (Irigoyen et al., 2010).

Les objectifs de ce volet du projet sont de déterminer l'influence (1) du pourcentage de récolte (la moitié ou la totalité) du feuillage et (2) du moment de cette récolte sur la croissance des plants d'ail des bois.

1.4.2.1 Hypothèses et prédictions

    A- Une période d'activité plus longue devrait permettre aux feuilles de fixer plus de carbone, qui sera transféré au bulbe pour assurer une meilleure croissance.

Ainsi, retarder le plus possible la récolte des feuilles, juste avant le début de la sénescence, permettra au plant de recouvrer plus rapidement sa taille initiale dans les saisons suivantes que si la récolte est hâtive (figure 1.3).

    B- Un pourcentage de récolte de surface foliaire plus élevé devrait réduire les réserves du bulbe (carbone et nutriments) et ralentir la croissance des années suivantes.

Ainsi, la croissance au cours des saisons suivantes sera plus forte si le pourcentage de surface foliaire récoltée est diminué (figure 1.3). Cette croissance améliorée pourrait être le résultat de la récupération des nutriments par le bulbe lors de la sénescence. La feuille restante continue aussi sa photosynthèse après la récolte d'une première feuille.
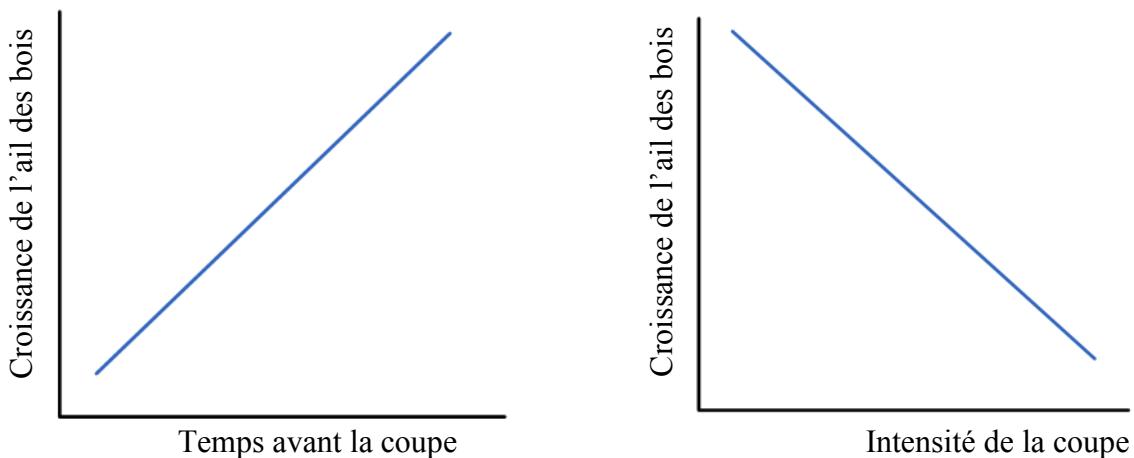
Figure 1.3 Prévision de la variation de la croissance de l'ail des bois selon le moment de la coupe des feuilles et l'intensité de cette coupe.

1.4.3 Densité

Les populations naturelles d'ail des bois ont souvent des densités élevées de l'ordre de 90 plants m^{-2} (Dagenais, 1985; Nault & Gagnon, 1993), mais peuvent atteindre, par endroit, une densité jusqu'à 350-400 plants m^{-2}. Dans un contexte de culture, on peut se demander si de telles densités ne nuisent pas au rendement de la population, en induisant une forte compétition pour les ressources entre les individus. Dans le cas de l'oignon (*Allium cepa*), la taille du bulbe et le nombre de feuilles augmentent lorsque la densité diminue. Toutefois, le rendement total maximal d'une culture, en masse produite par hectare, se situe à une densité intermédiaire (McGeary, 1985). Ritchey & Schumann (2005) ont testé l'effet de deux densités faibles (un plant par 15 ou 30 cm, soit respectivement 44 et 11 bulbes m^{-2}) sur la croissance et la survie de l'ail des bois et n'ont observé aucune différence. Cela suggère l'existence d'une densité minimale en deçà de laquelle une baisse n'a plus d'effet sur la croissance. À l'opposé, Nault & Gagnon (1993) ont observé une mortalité des plants adultes associée à une densité trop élevée, similaire aux densités observées en milieu naturel.

Les populations d'ail des bois sont particulièrement sensibles à la récolte. Nantel et al. (1996) estiment à 8% le taux maximum de récolte annuelle durable, soit le taux de récolte assurant une probabilité de 95% que la population survive aux 100 prochaines années. Nault & Gagnon (1993) prédisent quant à eux un déclin de populations à long terme dès que 10 à 15% des plants sont récolté par année, ou 5% si les conditions environnementales sont défavorables (ex. sécheresse). Ces estimations sont basées sur une récolte faite en prenant des individus « ici et là », plutôt qu'en récoltant complètement une portion de la population, comme le font la plupart des braconniers. Ces deux études ne prennent cependant pas en compte l'effet d'une variation de la densité de la population, suite à la récolte d'une partie de celle-ci, sur la croissance des individus restants. La baisse de densité ainsi causée pourrait accélérer la croissance des bulbes survivants, via une diminution de la compétition, aidant ainsi la régénération de la population après la récolte. Si cette amélioration de la croissance compensait en partie les pertes en termes de nombre d'individus grâce à une augmentation de la taille des plants et de leur potentiel reproducteur (sexué et asexué), les taux de récoltes maximums durables pourraient être en réalité plus élevés que ceux prédits dans ces études. D'ailleurs, suite à un fort pourcentage de récolte,

Rock et al. (2004) ont observé un taux de croissance supérieur à celui suivant une faible récolte, suggérant que la faible densité laissée par une forte récolte favorise la croissance des survivants. Ils ont toutefois noté que même après quatre ans, une population d'ail des bois n'a pas atteint les densités initiales suite à une récolte de 25% des individus. Le temps de régénération serait plutôt de l'ordre de 22 ans. Ils suggèrent donc un taux de récolte d'à peine 10% tous les 10 ans.

Les objectifs de ce volet du projet sont de (1) quantifier l'impact de la densité de plantation d'ail des bois sur sa croissance et ainsi estimer la densité permettant un rendement optimal de la culture, puis (2) de quantifier la régénération d'une population d'ail des bois suite à la baisse de densité causée par une récolte.

1.4.3.1 *Hypothèse et prédictions*

A- La compétition entre les plants devrait augmenter avec la densité des plants, ce qui aura un impact sur la croissance des plants individuellement et sur la croissance de la population.

La croissance du bulbe augmentera avec une diminution de la densité de plantation des plants. Le nombre de plants par m^2 augmentera plus rapidement dans les parcelles moins denses du fait que les plants plus gros se divisent et fleurissent davantage que dans les parcelles plus denses. Toutefois, une croissance maximale sera atteinte, à partir de laquelle une diminution supplémentaire de la densité sera sans effet (figure 1.4). De plus, la densité donnant la meilleure croissance par plant ne sera pas nécessairement celle générant le meilleur rendement global en termes de biomasse par surface cultivée.

Une récolte d'un pourcentage élevé de la population permettra une croissance plus rapide des plants et de la population (en termes d'effectifs) dans les années suivantes que suite à une récolte modérée. Par contre, le temps de régénération vers l'état d'origine sera plus court suite à une récolte modérée.

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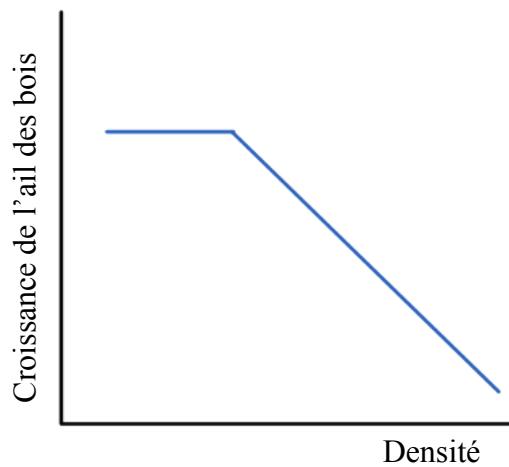
Figure 1.4 Prévision de la variation de la croissance de l'ail des bois selon la densité de la population.

Chapitre 2. Late canopy closure delays senescence and promotes growth of *Allium tricoccum*, a spring ephemeral.

2.1 Abstract

Wild leek (*Allium tricoccum*) is an edible forest spring ephemeral very popular in northeastern America. The plant takes advantage of the high light conditions in spring, before tree leaves unfold, to accumulate carbon reserves through photosynthesis. Although previous research did not consider light availability to influence wild leek growth, recent work reports delayed leaf senescence under constant light availability. This paper aims at establishing if tree canopy composition and phenology can influence wild leek growth through changes in its phenology and improvement of total light availability.

In a first experiment, bulbs were planted in 31 plots in southern Québec, Canada, under canopies varying in composition and densities. Light availability and tree phenology were measured along with other environmental conditions and their effect on wild leek growth was assessed with a redundancy analysis. Concomitantly, an eleven-year demographic study was performed on 18 wild leek plots in natural populations. Light availability and tree phenology were measured, along with plant number and size.

Higher light availability resulted in a better growth of wild leek. The plants acclimated to the higher light availability and postponed their senescence under late bud-bursting trees, which lengthened the photosynthetically active period. Better bulb growth and seed production were then achieved. Tree litter, micronutrient availability and soil temperature and moisture levels also influenced wild leek growth and survival. Tree leaf phenology thus has a strong impact on wild leek growth by modulating the length of the wild leek growing season and its photosynthetic capacity.

Keywords

2.2 Résumé

L'ail des bois (*Allium tricoccum*) est une éphémère printanière forestière comestible très populaire au nord-est de l'Amérique. L'ail profite de la période de pleine lumière au printemps avant la fermeture de la voûte forestière pour accumuler ses réserves de carbone par photosynthèse. Des études précédentes ont considéré la disponibilité de la lumière comme un facteur peu important pour la croissance de l'ail, mais des travaux récents ont révélé que la sénescence des feuilles est retardée lorsque la disponibilité de la lumière est constante. Ce projet a pour objectif de déterminer si la composition de la voûte forestière et sa phénologie peuvent influencer la croissance de l'ail des bois en modifiant sa phénologie et en améliorant les conditions de lumière.

Au sud du Québec, 31 parcelles d'ail des bois ont été mises en place sous diverses voûtes forestières variant en composition et densité. La disponibilité de la lumière et la phénologie de la voûte ont été mesurées, de même que plusieurs autres variables environnementales. Leur effet sur la croissance de l'ail des bois a été vérifié par une analyse de redondance. Parallèlement, une étude démographique a été menée sur 18 parcelles naturelles d'ail des bois pendant 11 ans. La lumière disponible et la phénologie de la voûte forestière ont été mesurées, de même que le nombre et la taille des plants.

Une forte disponibilité de lumière favorise la croissance de l'ail des bois. Les plants se sont acclimatés à la plus grande disponibilité de la lumière et la sénescence des feuilles a été retardée sous les arbres à débourrement tardif, ce qui a allongé la période d'activité photosynthétique. Le recouvrement par la litière, la disponibilité en micronutriments et la température et l'humidité du sol influencent aussi la croissance et la survie. La phénologie de la voûte forestière affecte donc la croissance de l'ail des bois en modifiant la durée de la période de croissance et la capacité photosynthétique de l'ail des bois.

Mots-clés

Voûte forestière; Culture en boisé; Humidité du sol; Lumière; Litière; Micronutriments; Phénologie; Éphémère printanière; Température du sol; Ail des bois

2.3 Introduction

Wild leek (*Allium tricoccum* Aiton), also commonly known as ramp, is a spring ephemeral geophyte that is very popular in the cuisine and folk medicine of the central Appalachian Mountains of North America (Davis & Greenfield, 2002). Both its bulb and leaves are edible. The plant takes advantage of the high light conditions between snow melt and canopy closure to accumulate photosynthate (Lapointe, 2001). The leaves unfold early in spring and senesce shortly after complete canopy closure. Anthesis occurs later in summer and seed production is completed in early autumn (Jones, 1979).

The light environment in spring varies greatly in deciduous forests, depending upon the tree species, with some flushing their leaves later than others, which can influence understory vegetation growth (Kato & Komiyama, 2002). Low light levels are often limiting for forest herbs, especially those that are adapted to take advantage of high light conditions in early spring (Rothstein & Zak, 2001a). Indeed, the length of the period between snow melt and canopy closure is known to influence growth and reproduction of spring flowering species, such as wake-robin or red trillium (*Trillium erectum* L.; Routhier & Lapointe, 2002), and the timing of canopy closure can influence photosynthetic capacity and carbon assimilate translocation patterns of understory herbs (Ida & Kudo, 2008). Early leafing, as is observed in spring ephemerals, is a common shade-avoidance strategy for understory vegetation (Lopez et al., 2008). Moreover, Rothstein & Zak (2001a) reported that wild leek has a higher photosynthetic capacity than a summer-green or a semi-evergreen species that is found in similar habitat, which is a typical response of plants that are adapted to high light conditions.

Light passing to the forest floor after canopy closure can also influence growth. Of the four medicinal forest herbs that were studied by Naud et al. (2010), three exhibited better growth under higher light conditions. American ginseng (*Panax quinquefolius* L.), another forest medicinal herb, also responded positively to higher light availability (Fournier et al., 2004). The presence of canopy gaps increases light availability, which is often beneficial to the understory vegetation (Canham, 1988b). However, experiments directly involving wild leek did not report a relationship between its growth and light availability (Vasseur & Gagnon, 1994; Delagrange et al., 2013), although they did not take

into account variation in tree leaf phenology. In a recent growth chamber experiment, under constant light and temperature, it was observed that wild leek senescence is delayed compared to its phenology under natural conditions (Bernatchez & Lapointe, 2012). This suggests plasticity in its phenology, which could possibly acclimate to the canopy phenology and provide a longer active period of photosynthesis and, therefore, a larger final size at the end of the season.

Forest canopy composition can also influence other environmental variables such as litter abundance, which in return can influence temperature and moisture in the uppermost soil layers (Facelli & Pickett, 1991; Barbier et al., 2008). A sparse canopy can also modulate the abundance of other herb species that could compete with wild leek for light and nutrients (Plue et al., 2013). In contrast, tree composition can affect, and can be influenced by, soil nutrient and water availability (Barbier et al., 2008; Lukac & Godbold, 2011; Thomaes et al., 2013). Therefore, different canopies may reflect different soil types, which could also affect wild leek growth.

The general objective of this research was to determine the influence of canopy composition on wild leek growth. A multivariate approach was used to take into account differences in soil variables, along with light conditions. Furthermore, we measured leaf chlorophyll a/b ratios and bulb $\delta^{13}C$ to confirm that wild leek acclimated to the light conditions prevailing in the different plots. Chlorophyll a/b ratios are known to increase under high light conditions (Dale & Causton, 1992). The value of $\delta^{13}C$ usually increases with photosynthetic activity, because RUBISCO discriminates less against ^{13}C relatively to ^{12}C as activity of the enzyme increases (Dawson et al., 2002; Bowling et al., 2008). We measured the $\delta^{13}C$ in the bulb as an indication of the variation of mean carbon fixation rate among plots, assuming that the carbon isotopic composition of the fructans and other organic compounds accumulated in the bulb would reflect the mean carbon fixation rate throughout the growing season. Leaf $\delta^{13}C$, on the other hand, mostly reflects intercellular carbon concentration at the time of leaf formation only (Dawson et al., 2002).

We posit that 1) higher light availability in spring should increase wild leek growth; bulbs under a sparse canopy or gaps should thus get bigger; and 2) a lengthening of the period of high light conditions prior to canopy closure should also favor growth. Wild leek

should postpone senescence under a forest cover that is composed of late bud-bursting species (e.g., *Fraxinus* spp., *Quercus rubra* L.). A first experiment with transplanted bulbs aimed to test the light component of the environment in sites that exhibited high light variability among each other, but moderate environmental variability in terms of nutrient availability, soil pH and soil moisture. A second experiment in natural populations aimed to determine if the effect of light availability on wild leek growth is also noticeable in natural stands, albeit with greater environmental variability.

2.4   Material and Methods

2.4.1   Wild leek planting

2.4.1.1   Study sites
Experiments were conducted in Estrie (Eastern Townships) and Basses-Laurentides (Lower Laurentian) regions of southern Québec, Canada. These provincial administrative regions exhibit similar climatic conditions. Estrie (plots are located 45°05' to 45°25' N, 71°20' to 71°60' W) has an average annual temperature of 4.1 °C and annual precipitation of 1144 mm, and accumulates 1637 degree-days over 5 °C each year. The Lower Laurentides (45°25' to 45°40' N; 74°00' to 74°10' W) have an average temperature of 5.0 °C, precipitation of 1065 mm, and 1866 degree-days over 5 °C (Environment Canada, 2013). Both regions are located close to the northern limit of wild leek's geographic range and encompass several large natural populations of the species (Jones, 1979; Dagenais, 1985).

2.4.1.2   Experimental plots
During spring 2011, we surveyed many public and privately owned forests and tree plantations to identify plot areas exhibiting divergent canopy openings, canopy gaps, and overstory tree species composition. Within 13 sites, 47 plot size areas were pre-selected. Soil samples were collected and sent (Agri Quanta Inc., St-Ours, QC, Canada) for nutrient (Mehlich III extraction and ICP-AES), water and buffer pH, CEC, and organic matter (loss-on-ignition) analyses. Of these pre-identified plots, those with low pH, severe mineral deficiency or toxicity were excluded in favor of those plots where conditions were suitable for wild leek growth; 31 were retained, based on their accessibility and soil richness and are detailed in the "transplants" column of Table 2.1. The "Natural populations" plots also

detailed in Table 2.1 will be presented later. The exact positions of the plots remain undisclosed to prevent unauthorized harvesting.

Wild leek bulbs were harvested from a single site on May 30th, 2011. All bulbs were *Allium tricoccum* var. *tricoccum* (Jones, 1979). Bulbs were sorted and medium-size bulbs were replanted together in a nearby forest to complete their senescence.

Transplantation was conducted during the week of August 22nd, 2011. Bulbs were excavated, floral scapes (elongated, leafless flowering stems) were removed and bulbs were maintained in plastic bags in the dark under cool temperatures during transport. On each selected plot, a 1×3 m plot was delimited and tree saplings directly over it were cut. The soil was plowed to a 15-cm depth and fertilizer was applied following the recommendations of Bernatchez et al. (2013). The soil amendments consisted of 3000 kg ha^{-1} of gypsum (Uncalcined Gypsum Products, $CaSO_4$, Georgia-Pacific Gypsum Corporation, Atlanta, GA, USA) and 550 kg ha^{-1} of N-P-K fertilizer (5-10-8 from a mixture of Bio-Jardin 4–3–6 [N–P_2O_5–K_2O], which also contained 3 % Mg, and fossil bone 0–13–0, McInnes Natural Fertilizers Inc., Stanstead, QC). In each plot, 90 bulbs were planted at a depth of 5 cm and spaced 15 cm apart. Four plots contained only 85 bulbs, because there were not enough bulbs of the proper size available from the harvested site to complete each plot. Natural litter was set aside prior to plowing and returned to the plots after planting was completed. Weeding was performed during plot establishment and after wild leek senescence in 2012 and 2013.

Table 2.1 Main tree species composing the canopies above 30 wild leek transplant plots and 17 natural wild leek locations in the Parc national de la Yamaska, Québec.

Main tree species composing the canopy	Number of plots	
	Transplants	Natural populations
Acer saccharum Marshall[†]	11	9
Betula papyrifera Marshall	1	-
Betula alleghaniensis Britton	2	-
Carya cordiformis (Wangenh.) K.Koch	2	-
Carya ovata (Miller) K.Koch	-	1
Fagus grandifolia Ehrhart	3	-
Fraxinus sp.	-	2
Fraxinus americana L.	1	-
Fraxinus pennsylvaniva Marshall	1	-
Fraxinus nigra Marshall	1	-
Juglans nigra L.[‡]	2	-
Ostrya virginiana (Miller) K.Koch	1	1
Populus grandidentata Michaux	1	-
Quercus rubra L. [‡]	2	-
Tilia americana L. [†]	2	3
Ulmus sp.	-	1
Total	**30**[*]	**17**[**]

Notes:
[†] Four plots labeled '*A. saccharum*' and one plot labeled '*T. americana*' were actually in canopy gaps.
[‡] *J. nigra* and *Q. rubra* plots were located in   tree plantations.
[*] A 31st plot in a *F. americana* plantation was abandoned in 2012 because of severe herbivore grazing.
[**] An 18th plot in natural populations was abandoned due to recurrent flooding.

2.4.1.3   Wild leek growth measures

Growth of wild leek was measured during the 2012 and 2013 seasons, and included total leaf width (TLW; in early May, with a ruler), bulb width (early July, after complete leaf senescence, with a caliper), number of bulbs (early May), number of flowering scapes per plot (early July), and seed number per bulb (end of August). Total leaf width has been successfully correlated with plant biomass (Nault & Gagnon, 1993).

Weekly surveys were performed in all plots, during which we counted the number of plants in each of the following phenological stages: 1 = leaf emergence; 2 = leaf unfolding; 3 = fully expanded leaves; 4 = beginning of senescence with < 25% of leaf area senesced; 5 = advanced senescence with > 25% leaf area senesced; 6 = fully senesced leaves (Figure 2.1). Surveys were converted to percentages of individuals at a given stage. Since our objective was to assess the duration of wild leek photosynthetic activity and to transform these phenology surveys into a single value that was usable in linear correlation and redundancy analysis, we developed a *Wild Leek Phenology Index* (WLPI), as follows:

$$WLPI = \sum_{i=1}^{n} \left(N_i (S_{3i} + 0.595 S_{4i} + 0.255 S_{5i}) \right) \qquad \text{Eq. 2.1}$$

where *n* is the number of surveys that were performed during the growing season; N_i is the number of days accounted for by the survey, i.e., from the middle of the period between the $(i-1)^{th}$ and the ith surveys to the middle of the period between the ith and the $(i+1)^{th}$ surveys (the first and last periods respectively started or ended on the first and last surveys); S_{3i}, S_{4i} and S_{5i} are respectively the percentages of wild leek individuals at phenological stages 3, 4 and 5 at the ith survey. Spring ephemeral leaves attain their maximum photosynthetic rate at the beginning of stage 3 (Lapointe, 2001). During stages 4 and 5, photosynthetic capacity drops to 59.5 and 25.5 % of the maximum capacity that is recorded during stage 3, based on data that have been reported previously (Bernatchez & Lapointe, 2012); hence, the inclusion of the constants in equation 2.1. It should however be stressed that the measures from Bernatchez & Lapointe (2012) were taken in growth chambers providing constant light and temperature; the declining light and rising temperature usually observed in deciduous forests during spring may affect the variation in photosynthetic capacity, thus modifying the constants used in equation 2.1. Unfolding leaves usually act as C sinks for

the spring ephemeral *Erythronium americanum* Ker-Fawl. (Gandin et al., 2011); thus, stages 1 and 2 were not included. The last survey took place immediately following complete leaf senescence.

In order to confirm that plants had acclimated to the local plot light environment, we analyzed leaf chlorophyll a/b ratios (chl a/b) and bulb stable carbon isotopic composition ($\delta^{13}C$) in 2013 (Year 2). We randomly sampled four leaves from different plants in each plot, after canopy closure, and kept them frozen at -15 °C until measurements could be made. Following acetone extraction, chlorophyll absorbance of the supernatant was measured by spectrophotometry and quantified using the equations of Porra & Grimme (1974).
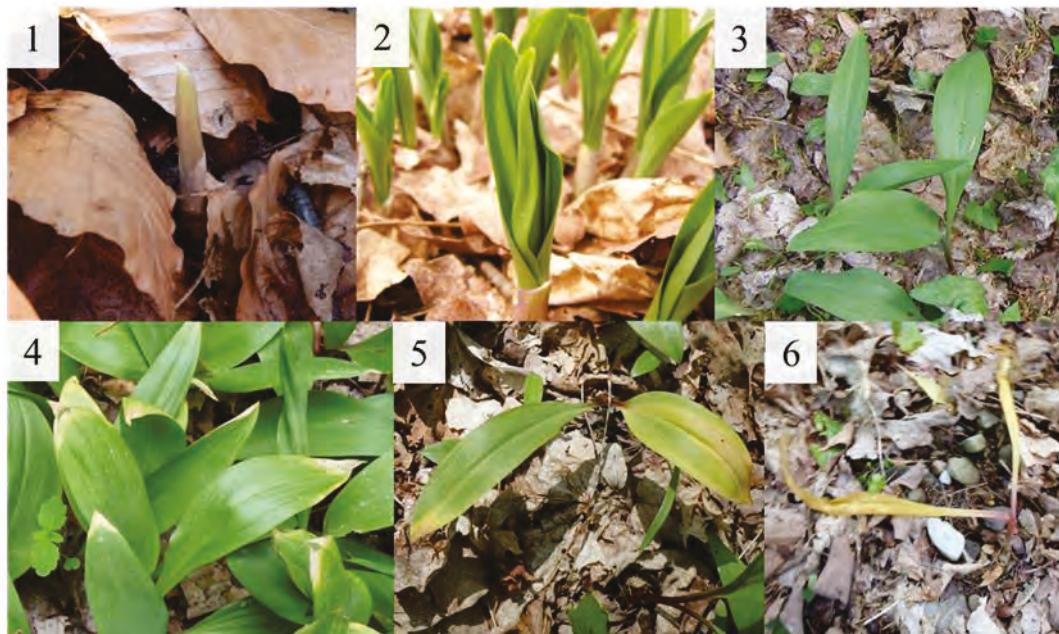
Figure 2.1. Leaf phenological stages of wild leek as used in the calculation of WLPI. 1 = leaf emergence; 2 = leaf unfolding; 3 = fully expanded leaves; 4 = beginning of senescence with < 25 % of leaf area senesced; 5 = advanced senescence with > 25 % leaf area senesced; 6 = fully senesced leaves.

We measured the $\delta^{13}C$ in the bulb as an indication of the variation of mean carbon fixation rate among plots throughout the growing season. We harvested four bulbs per plot after complete leaf senescence in early July. They were kept refrigerated for less than 4 days, dried at 70 °C for 48h, and ground to powder in a mortar with a pestle. Samples encapsulated in tin were sent to the Stable Isotope Facility at the University of California, Davis, for ^{13}C analysis (PDZ Europa ANCA-GLS elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer; Sercon Ltd., Crewe, Cheshire, UK).

2.4.1.4 Light estimation

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

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

Total light reaching the plot during the growing season of wild leek was modeled by multiplying the calculated daily solar radiation with the number of days that each photograph represented. We defined the period between complete snowmelt (19 April) and complete leaf senescence (15 June) as the 2013 growing season.

Canopy phenology status was quantified by estimating the mean leaf width of the main tree species surrounding each plot. We developed a *Canopy Closure Index* (CCI) using the formula:

$$CCI = \sum_{i=1}^{n} \left(\left(\frac{L_i}{L_{max}} \right) \times N_i \right) \qquad \text{Eq. 2.2}$$

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

2.4.1.5 Other abiotic factors

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

Four leaves were randomly sampled in each plot in 2013 for nutrient compositional analysis. They were dried at 70 °C and ground to powder in a mortar with a pestle. N and S were measured with an automated Leco CNS-2000 dry combustion analyzer, whereas Al, Ca, Cu, Fe, K, Mg, Mn, P and Zn were quantified by HNO_3-$HClO_4$ digestion, followed by ICP-AES (Léon-Étienne Parent's laboratory, Laval University, Québec, QC).

2.4.2 Natural populations

In the Parc National de la Yamaska of southern Québec, Canada, eighteen 1 m² plots were randomly distributed in 5 different wild leek populations under varying canopy composition (Table 2.1). These plots have been followed throughout the *Ecological Integrity Monitoring Program* for the last 11 years (Parc Québec Network, 2014). The

number of bulbs, number of leaves per bulb, flowering frequency and seed production were recorded each year. Analyzed data covered seasons 2003 to 2013. In spring 2013, we took hemispherical photographs of canopies following the same method that was employed in the transplant plots. For logistical reasons, we could only take two series of photographs: a first one before tree bud burst and a second one after complete canopy closure. However, wild leek and canopy phenology were followed weekly until complete senescence. We thereafter modeled total light in a manner similar to that used in the transplant plots, by simply progressing from maximum to minimum light using the percentage leaf opening (L_i/L_{max} in eq. 2.2).

2.4.3 Statistical analyses

2.4.3.1 Redundancy analysis

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

2.4.3.2 Compositional analysis

Nutrient data were first analyzed separately, given that there were too many nutrient variables to include them all in the RDA. Compositional data such as mineral concentrations add up to unity, which implies that the variation of one mineral inevitably affects the concentration of the others. Nutrient balance analysis using the isometric log-ratio transformation (*ilr*) was applied to the nutrient data, as suggested by

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Parent et al. (2013b), using the equation, $ilr_i = \sqrt{\frac{n_i^+ n_i^-}{n_i^+ + n_i^-}} \, ln \frac{g(c_i^+)}{g(c_i^-)}$, where n_i^+ and n_i^- are respectively the number of nutrients in the numerator and denominator, and $g(c_i^+)$ and $g(c_i^-)$ are respectively the geometrical means of the nutrients in the numerator and denominator of the ratio. This method of compositional analysis was developed to overcome the bias caused by the dependencies among the mineral concentrations. See Parent et al. (2013b) for a complete description of the rationale for using nutrient balances rather than individual nutrient concentrations or two by two ratios. We used the *compositions* package (Boogaart et al., 2013) in *R* 2.15.1 (R Core Team, 2012). Selected orthogonal mineral balances are shown in Table 2.2, the choice of which was inspired by Parent et al. (2013a). We did not include C to avoid redundant relationships with photosynthetic activity, which, according to our hypothesis, would be influenced by light conditions. Multiple regression by forward selection, combined with 10,000 permutations, was conducted in the *packfor* package (Dray et al., 2011), using the same response variables as the RDA (except WLPI). This allowed us to select the most important nutrient balances that were to be included in the RDA as additional environmental factors. Leaf nutrient balances were used as proxies for soil nutrient availability.

2.4.3.3 *Natural populations*

Given the high mortality rate of seedlings and the great variability in annual seed production (Nault & Gagnon, 1993), the number of mature bulbs appeared to be a more stable indicator of population growth. Thus, only two- and three-leafed bulbs were considered in all analyses from the natural populations. We calculated, using linear regression, the annual rate of population growth for each plot from 2003 to 2013. Pearson product-moment correlations (*r*) were calculated between the different light variables and annual population growth, together with the individual plant variables that were measured in 2013.

Table 2.2 Foliar nutrient balances used for the computation of the ilrs, and their correlations with soil pH (in water) in transplant plots of wild leek.

ilr† : mineral balances	r ‡
[Al | Fe,Mn,Zn,Cu,S,Mg,Ca,K,P,N]	0.386 *
[Fe | Mn]	0.793 ***
[Zn | Cu]	- 0.777 ***
[Fe,Mn | Zn,Cu]	- 0.719 ***
[Fe,Mn,Zn,Cu | S,Mg,Ca,K,P,N]	- 0.828 ***
[Mg,Ca,K | S,P,N]	0.623 ***
[Mg,Ca | K]	- 0.435 *
[Mg | Ca]	0.831 ***
[P | N]	0.172 n.s.
[S | P,N]	- 0.453 *

Notes:
† Components on the left and on the right side of the mineral balance are respectively the numerator and the denominator of the isometric log-ratio (*ilr*).
‡ Correlation between the mineral balance and soil pH. *P*-value: n.s.: non-significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. A positive relationship between a balance and pH means that at higher pH, elements on the left side of the balance are more abundant in the leaf tissues relative to those on the right side of the balance. A negative relationship thus indicates that at higher pH, elements on the left side of the balance are less abundant in the leaf tissue relative to those on the right side.

2.5 Results

2.5.1 Transplant plots

2.5.1.1 Nutrient analysis

Foliar nutrient concentrations from the transplant plots are presented in Figure 2.2. Mineral balances (Table 2.2) were calculated using these foliar nutrient concentrations, and of the 10 balances, four were retained in the forward selection model as having a significant effect on wild leek growth (Table 2.3). The four balances were first included in the initial RDA (not shown). The [Mg | Ca] and [Zn | Cu] vectors were at angles close to 90° to the response variables, i.e., they were orthogonal to the latter. Given that they were uncorrelated with the response variables, the nutrient vectors did not provide more information, except for a strong link with pH (respectively, r = 0.831, P < 0.001 and r = - 0.777, P < 0.001), which was only evident along RDA axis 4 (not shown). These ratios were subsequently removed from the RDA. Therefore, only the two other significant ratios were included in the final RDA, i.e., [Fe,Mn | Zn,Cu] and [Mg,Ca,K | S,P,N].
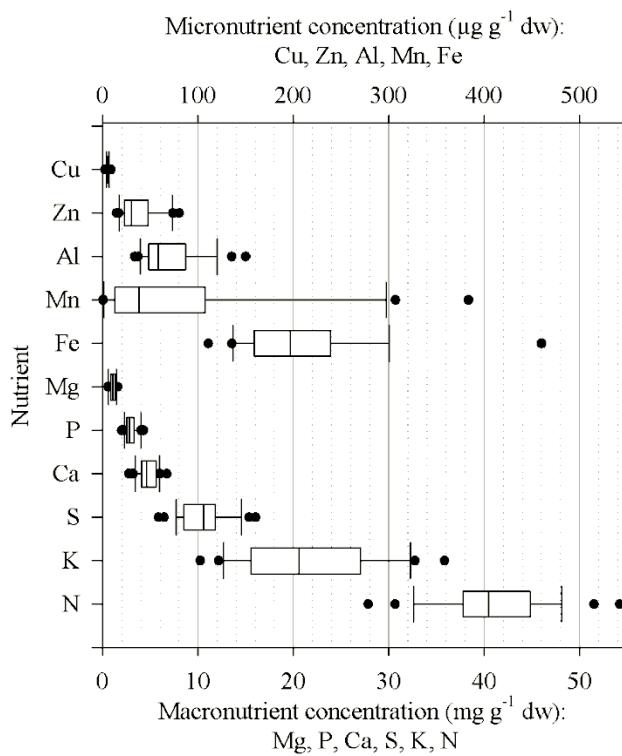
Figure 2.2 Variability in foliar nutrient concentrations of wild leek two years after planting and fertilization. 10th, 25th, 50th, 75th and 90th percentiles are presented as box and whiskers. Values below and over the 10th and 90th percentiles are presented as dots.

Table 2.3 Forward selection of mineral balances in multiple regression versus four wild leek growth response variables in the transplant plots, based on 10,000 permutations.

Mineral balances	Cumulated adjusted R^2	P-value
[Fe,Mn	Zn,Cu]	0.198	0.001
[Mg	Ca]	0.287	0.006
[Mg,Ca,K	S,P,N]	0.334	0.033
[Zn	Cu]	0.387	0.029

Note:
Mineral balances presented had a significant effect on wild leek growth.
Wild leek growth response variables included mean bulb width, total leaf width, average seed number produced per bulb, and total bulb number per plot.

2.5.1.2   Redundancy analysis

Triplots of the RDA that was performed on the data from the transplant plots are presented in Figure 2.3. The RDA ordination was significant (permutation test, $F_{10,\ 19} = 3.929$, $P < 0.001$; adjusted $R^2 = 0.502$); 50.2 % of the variation in the response data (i.e., wild leek growth) was explained by variation in the environmental data. Four canonical axes were significant ($P < 0.05$) and respectively explained 30.3, 9.5, 5.7 and 3.5 % of the constrained variance. Axis 4 is excluded from Figure 2.3 because it accounted for < 5% of the variance and did not reveal any new relationships between response and environmental variables. Only environmental variables that exhibited strong relationships (either positive or negative) with the response variables are presented. Weed cover was not linked to any of the variables and was removed from the analysis.

Bulb width and seed production per bulb varied mostly along the first canonical axis (Fig. 2.3a). The three environmental factors that varied most along the same axis were total light, CCI and the [Fe,Mn | Zn,Cu] ratio, indicating that bulbs were bigger and seed production was higher under late-closing canopies (i.e., low CCI), where the total light that was received during the growing season was higher, and where Zn and Cu were more readily available compared to Mn and Fe. Soil moisture also varied mostly along axis 1. However, since its vector varied in the opposite direction from the bulb width and seed production vectors along axes 2 and 3 (Fig. 2.3c), we cannot confirm a positive relationship between moisture and these two response variables. Average soil temperature exhibited a weak positive relationship with bulb width (Fig. 2.3c); in the last week of April, plots with a higher temperature had a higher percentage of leaves in stage 2 (unfolding; $r = 0.589$; $P = 0.002$; data not shown). Mean temperature throughout the growing season and soil water content in all surveys are presented in Annex 2. Soil water content data from the first survey (April 21-22) were discarded due to an error in the programmation of the TDR, but we suspect near-saturation soil water content in all plots because of the recent melting of the snow cover.
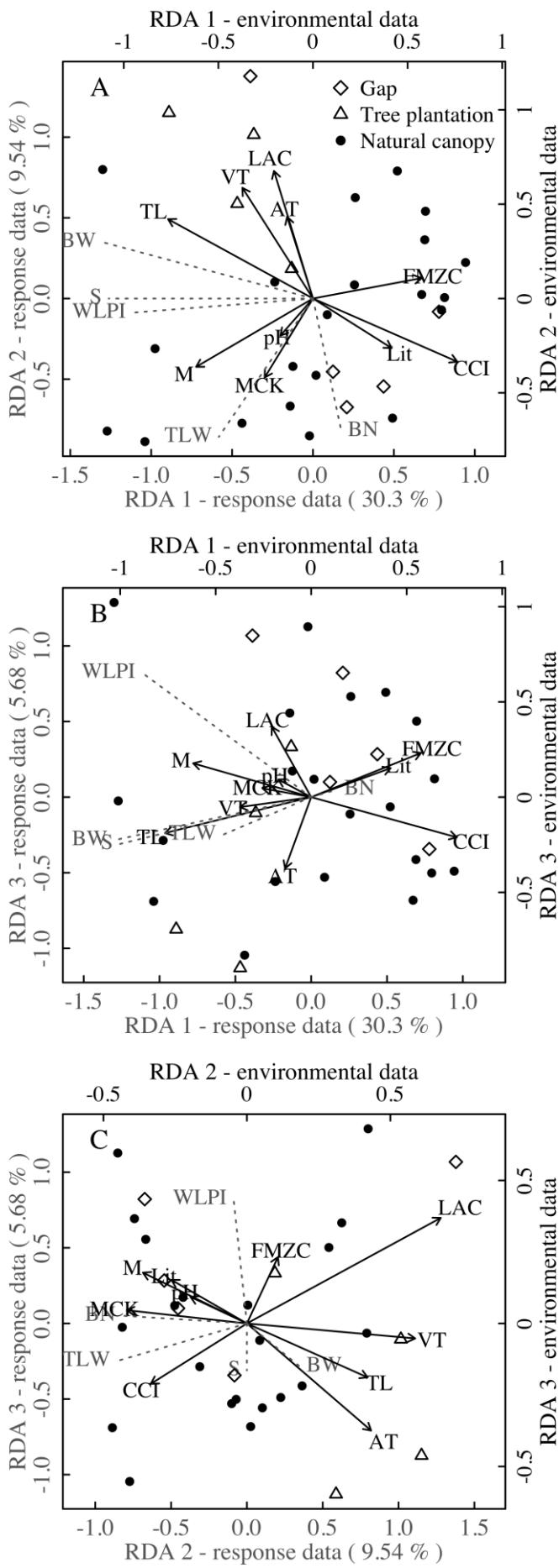
Figure 2.3 Redundancy analysis of the wild leek growth variables (gray, dashed lines) constrained by environmental variables (black, full lines). Each dot represents a transplant plot. BN = bulb number per plot; TLW = total leaf width per plant; S = seed number per bulb; BW = bulb width per plant; WLPI = wild leek phenology index; Lit = litter cover; M = soil moisture; TL = total light during the growing season; LAC = light availability after canopy closure; AT = average soil temperature; VT = soil temperature variance; CCI = canopy closure index; pH = soil water pH; FMZC = [Fe,Mn | Zn,Cu] foliar nutrient balance; MCK = [Mg,Ca,K | S,P,N] foliar nutrient balance.

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There was a negative relationship between the bulb number and light availability after canopy closure, average temperature and its variance (Fig. 2.3a,c); bulb division and survival may be affected by high and fluctuating temperature under sparse canopy. In contrast, the vector for the litter cover percentage was oriented in the same direction as that for bulb number. Litter could be considered, therefore, as being positively linked to bulb number. WLPI and CCI varied in opposite directions along axes 1 and 3, but not along axis 2. However, variation in these variables along axis 2 was marginal. High soil moisture also seemed to favor a higher WLPI (Fig. 2.3a-c).

The [Mg,Ca,K | S,P,N] nutrient balance was linked to TLW on axes 1 and 2, meaning that plants richer in Mg, Ca and K compared to S, P and N produced larger leaves. Soil pH had a strong influence on many nutrient ratios (Table 2.2), but varied mostly along axis 4 and was uncorrelated with the response variables (data not shown). Soil pH ranged from 4.09 to 6.72.

2.5.1.3   Acclimation to light conditions

In order to formally test the two components of the main hypothesis, i.e., that 1) a sparse canopy and 2) a late canopy closure would favor wild leek growth, the relations between bulb width and total light availability for the growing season, daily light availability after canopy closure and CCI were tested (Fig. 2.4). Bulb width is the best indicator of annual growth of individual wild leek plants and was thus selected as the main response variable. Total light during the growing season explained 62 % of bulb-width variance (Fig. 2.4a). The two sub-components that influenced the most total light that was received throughout the season are presented in Figures 2.4b and c. Bulbs appeared to be unaffected by the light level after canopy closure (Fig. 2.4b), but grew bigger under a late-closing canopy (Fig. 2.4c). Photosynthetic rates could also be affected by the percentage of total light that was direct rather than diffuse (percentage direct light). There was a non-significant tendency toward a positive relationship between bulb width and percentage direct light during the growing season (not shown; $r = 0.33$; $P = 0.08$), but there was no relationship between bulb width and percentage of direct light after canopy closure (not shown; $r = 0.14$; $P = 0.47$).
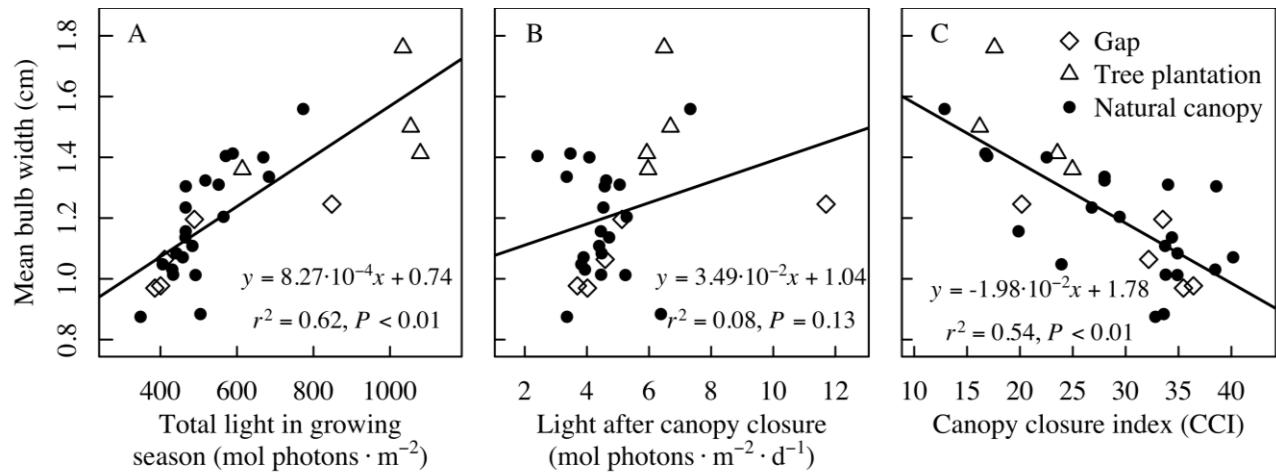
Figure 2.4 Linear response of mean bulb width versus a) total light availability during the growing season and two light parameters that influence total light availability in the transplant plots, i.e., b) light after canopy closure and c) the canopy closure index. Light after canopy closure is estimated from hemispheric photographs and includes both direct and diffuse light.

Of the plots within canopy gaps, only one benefited from higher light conditions after canopy closure. Plots in the tree plantations received more light, grew bigger bulbs, and their canopy closed later, compared to the plots located in natural stands. WLPI and CCI were negatively correlated (Fig. 2.5); wild leek in plots under a late-closing canopy (low CCI) had a longer active period before senescence (high WLPI). This relationship was significant for the two years that these variables were recorded. RDA also displayed this strong negative relationship between WLPI and CCI (Fig. 2.3 a, b).

RDA indicated that the percentage of litter cover and the [Fe,Mn | Zn,Cu] ratio exerted effects that were opposite to that of total light (Fig. 2.3a, b, c). To confirm that the observed responses in wild leek growth actually originated from light rather than from the presence of a thinner litter layer or from nutrient availability, we analyzed chlorophyll a/b ratio and the stable carbon isotopic ratio ($\delta^{13}C$). Both ratios were significantly correlated with total light and mean bulb width (Fig. 2.6). Plants growing under high light conditions produced leaves with higher chl a/b and higher $\delta^{13}C$ ratios, indicating acclimation to higher light conditions and enhanced RUBISCO activity over the entire growing season; consequently, plants with higher chl a/b and $\delta^{13}C$ ratios produced larger bulbs. This response was not due to a thinner litter layer or to enhanced soil Zn and Cu availability, but most likely in response to light availability.
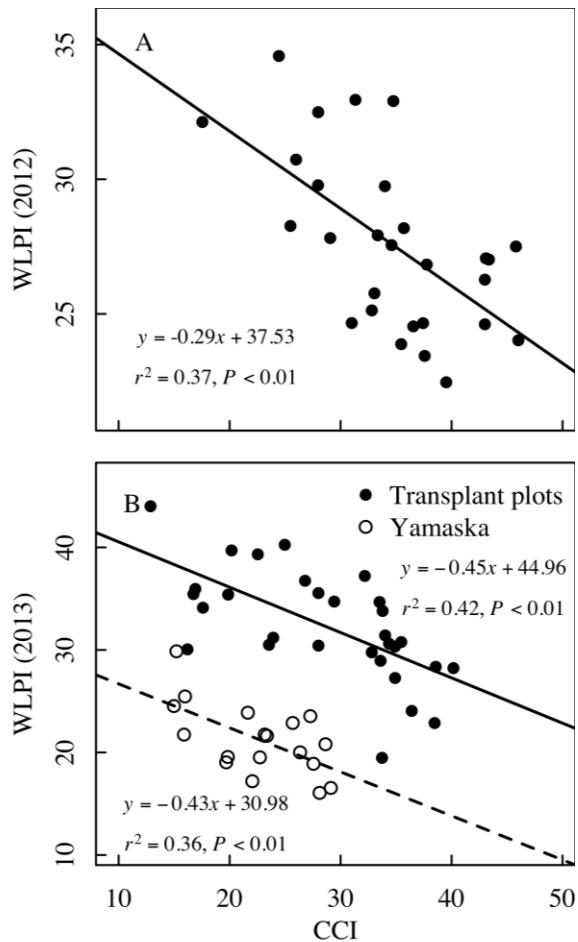
Figure 2.5 Linear relationship between Wild Leek Phenology Index (WLPI) and Canopy Closure Index (CCI) in transplant plots and natural populations (Yamaska). Phenological observations on transplant plots were recorded in 2012 and 2013. The three data sets were analyzed separately, because the surveys were not performed over the exact same periods that influenced WLPI and CCI values.

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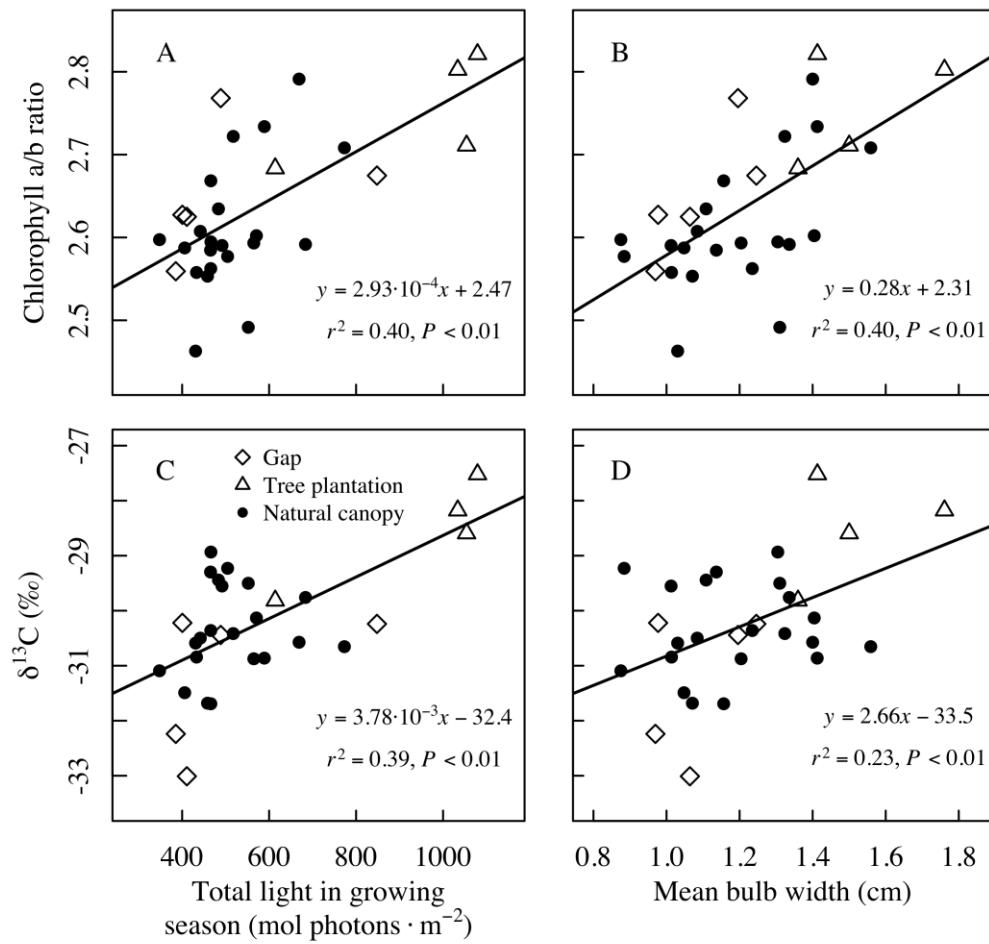
Figure 2.6 Chlorophyll a/b ratio (a, b) and stable carbon isotopic composition (δ^{13}C; c, d) exhibited a significant linear relationship with total light reaching the plot during the growing season (a, c) and the mean bulb width (b, d).

2.5.2   Natural populations

Natural populations surveyed under the *Ecological Integrity Monitoring Program* in the Parc national de la Yamaska revealed a relatively constant increase in the number of mature bulbs ($\geq$ 2 leaves) up to 2013 (Fig. 2.7). Population growth ranged from 0.75 to 9.75 new mature bulbs per year ($\bar{x} = 4.16$, $s = 2.81$). The number of one-leaf plants was also fairly constant, except in 2012 and 2013, which had followed two good years of seed production. The only plot that exhibited a progressive reduction in number of bulbs had suffered recurrent flooding in spring and, thus, was not included in the growth analysis. As expected, the numbers of floral scapes and seeds fluctuated greatly from year to year (Fig. 2.7).
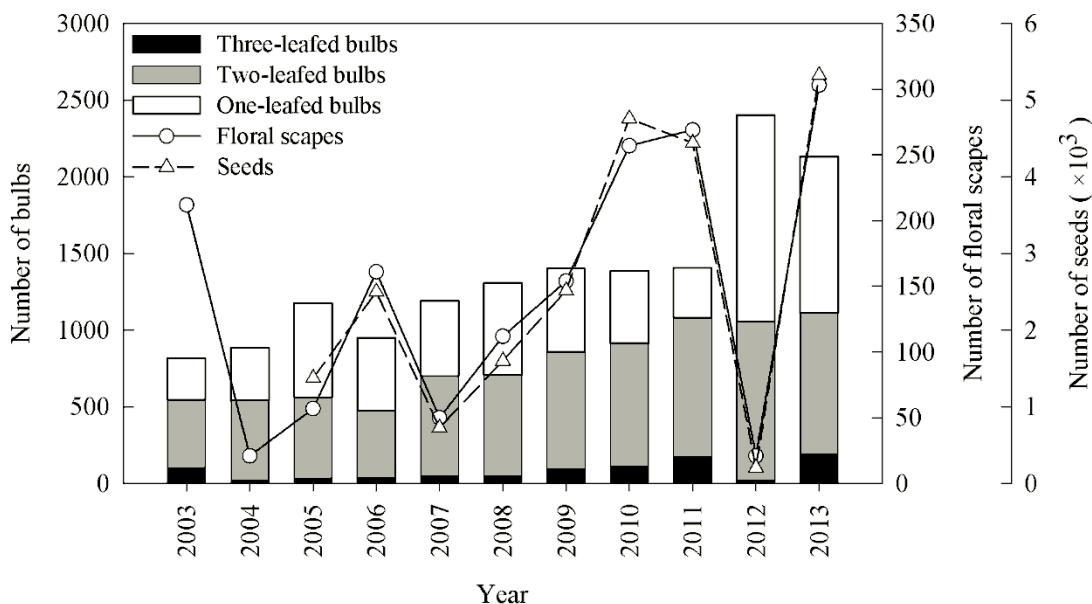
Figure 2.7 Demographic evolution of natural wild leek populations within the Parc national de la Yamaska. Data that are presented include all plots.

There was no positive relationship between light conditions and wild leek individual and population growth variables in the natural populations within the Parc national de la Yamaska (Table 2.4). Population growth was positive and varied widely among plots, but total light and light availability after canopy closure did not explain a significant part of this variation. These light variables and CCI also failed to explain the number of bulbs in 2013, the mean number of leaves, and seed production per bulb, except for a significant positive

relationship between number of seeds that were produced per bulb and total light throughout the season. There was also a significant positive correlation between annual population growth and WLPI ($r^2 = 0.31$, $P = 0.02$), but not directly between annual population growth and CCI ($r^2 = 0.076$, $P = 0.283$; Table 2.4). WLPI increased with CCI, as previously reported in transplant plots (Figure 2.5b). Since litter cover was uniform (almost always 100 % coverage) in natural populations across the Parc National de la Yamaska, its effect on wild leek growth could not be tested.

Table 2.4 Pearson correlations between light availability and wild leek growth in natural populations that were located within the Parc national de la Yamaska, Québec.

	Total light throughout the season (mol photons m^{-2})		Light after canopy closure (μmol m^{-2} s^{-1})		Canopy closure index (CCI)	
	r	P	r	P	r	P
Population growth (bulb yr^{-1})	0.318	0.214	0.081	0.758	0.276	0.283
Bulb number per plot	0.377	0.135	0.089	0.733	0.393	0.118
Mean leaf number per bulb	0.051	0.845	- 0.233	0.368	- 0.218	0.645
Mean seed number per bulb	**0.522**	**0.032**	- 0.141	0.590	- 0.111	0.660
Wild leek phenology index (WLPI)	0.025	0.925	- 0.274	0.754	**0.604**	**0.008**

Notes:
All data are from 2013, except for population growth (bulb yr^{-1}), which covers 2003 to 2013. Values in bold indicate a significant linear correlation ($\alpha < 0.05$).

2.6 Discussion

2.6.1   Light

More light reaching the soil during the growing season translated into more growth of wild leek. Canopy phenology is the sub-component of the light environment that explains most of the difference in bulb growth and seed production. Delayed canopy closure resulted in a longer period of high light conditions and allowed more carbon accumulation in the bulb and a greater investment in seed production. This enhanced productivity was most likely a result of the acclimation of wild leek phenology to canopy phenology; leaf senescence was delayed under a late bud-bursting canopy. Previous studies regarding the influence of the light environment on wild leek growth did not consider tree phenology, which could explain why no effect of light was detected (Vasseur & Gagnon, 1994; Delagrange et al., 2013). The effect of tree leaf phenology on forest herb growth has been seldom studied, but Routhier & Lapointe (2002) found that *Trillium erectum* growth and reproduction were enhanced when there was a longer period of high light conditions prior to canopy closure. Seedlings of *Acer mono* (*A. pictum* subsp. *mono* [Maxim.] H.Ohashi) also were shown to have greater growth under a late-closing canopy (Seiwa, 1998). Further, it should be considered that southern Québec is located close to the northern limits of wild leek's geographic range (Jones, 1979). The time period between snow melt and canopy closure is shortened in sugar maple forests that are growing at more northerly latitudes (Routhier & Lapointe, 2002). This may contribute to explaining the critical importance of canopy phenology for wild leek growth that we recorded in the present study.

Previous studies of wild leek have mostly considered light as a marginal parameter (Vasseur & Gagnon, 1994; Delagrange et al., 2013). Yet, light has been reported to affect other spring forest herbs. For example, Naud et al. (2010) found a significant positive effect of light after canopy closure on the performance of the forest herbs black cohosh (*Actaea racemosa* L.), wild ginger (*Asarum canadense* L.), and bloodroot (*Sanguinaria canadensis* L.), but not on blue cohosh (*Caulophyllum thalictroides* [L.] Michaux). Ida & Kudo (2008) reported an effect of shading on reproduction and carbon allocation in *Trillium apetalon* Makino. In the present study, variation in the chl a/b ratio and in $\delta^{13}C$ as a function of total light reaching the plot during the season confirmed that wild leek did acclimate to the light

conditions and that mean photosynthetic rates increased with light availability, as would be expected for leaves that are acclimated to higher light conditions (Nobel, 1991). This acclimation to light availability could explain the increased bulb size that was reported for wild leek plots receiving more light throughout the growing season, along with delayed leaf senescence in these plots. Phenotypic plasticity such as observed for leaf phenology and photosynthetic capacity can help provide a relatively high fitness across various environments without high maintenance cost (Griffith & Sultan, 2012).

Gaps in the canopy did not have the expected positive effect on light availability. Of the five plots that were located within a gap, four plots had light conditions similar to the other plots under closed canopies. Those plots were positioned directly under the gap center. Given that the sun does not pass at the zenith at a latitude of 44° N, plots under the gaps did not receive more direct light. Indeed, according to Canham (1988a), the point receiving the most light under a 10-meter diameter gap located at these latitudes would be located 5 m outside the vertical projection of the gap. Moreover, the percentage of total light transmitted as direct light only marginally affected growth of wild leek. Although we would expect a shade avoiding herb such as wild leek to respond more to direct than to diffuse light, most of light available in temperate deciduous forests during spring is indeed direct (Brantley & Young, 2009). Wild leek may be light saturated during most of its growing season, until canopy closure, hence the absence of a significant relationship between growth and percentage of direct light. The fifth gap plot received much more light after canopy closure than did the other plots, but this surplus of light did not translate into enhanced bulb width. There was no reason to remove this apparent outlier from the correlation that is presented in Figure 2.4b, but doing so would change the relationship from non-significant to barely significant ($r^2 = 0.156$, $P = 0.034$), suggesting that increased light availability under a sparse canopy could have a positive and significant, albeit marginal effect on wild leek growth. It is surprising for a spring ephemeral that the growth of its bulb would be influenced by the amount of light passing through the canopy following its closure. Therefore, ongoing photosynthesis during leaf senescence could be sufficiently important to influence final reserve accumulation in the bulb.

2.6.2 Temperature

Wild leek population growth (bulb number) was higher when soil temperature was lower and exhibited smaller daily fluctuations. Likewise, Bernatchez & Lapointe (2012) found that wild leek bulb growth and net photosynthetic rates were higher under cool temperatures, which were representative of early spring compared to warmer late-spring temperatures. Enhanced bulb growth could explain the increase in bulb number, either through greater survival or increased frequency of bulb division. In the present study, we reported a positive link between mean soil temperature and bulb width. This apparently contradictory result might be due to higher air temperatures in spring-time melting the snow more rapidly, which would lengthen the wild leek growing season. The higher soil temperature observed in our experiment would be a consequence of higher air temperature and earlier snowmelt. Indeed, leaves started to unfold earlier in plots that were warmer in late April (data not shown). Temperature has been previously identified as a critical factor triggering leaf unfolding and senescence of spring ephemerals (Lapointe, 2001; Yoshie, 2008; Bernatchez & Lapointe, 2012), yet canopy phenology had a stronger influence than temperature upon the overall length of the epigeous period during the present study, according to the RDA (Fig. 2.3). However, the light environment could have influenced soil temperature. Soil temperature mean and variance were positively linked to minimum light levels that were transmitted after canopy closure, which is mostly a function of canopy density. The forest canopy is known to stabilize air and soil temperature and moisture (Morecroft et al., 1998), which could benefit wild leek. For instance, Diaci et al. (2012) reported higher *Allium ursinum* L. cover under a dense canopy compared to canopy gaps, which differed in terms of light availability after canopy closure.

2.6.3 Moisture

Soil moisture emerged as the most important environmental factor explaining total leaf width variation and ramet demography of wild leek in an experiment that was conducted by Vasseur & Gagnon (1994). We observed a similar positive link between soil moisture and leaf width, but not with the number of bulbs or the size of bulbs. Another spring ephemeral, *Erythronium japonicum* Decaisne, exhibited a reduced photosynthetic rate in a dry environment under an open canopy, which could be overcome by increasing the relative humidity of the air (Sawada et al., 1997). Axmanova et al. (2011) reported a small, but

significant positive influence of soil moisture levels on overall herb layer productivity in central Europe oak forests. Low air relative humidity and soil water content can also reduce stomatal conductance, thus reducing intercellular carbon dioxide concentration and increasing $\delta^{13}C$ (Scheidegger et al., 2000). Soil moisture and light availability being poorly related in our experiment (Fig. 2.3), the variation in soil moisture among plots may have induced additional variation in $\delta^{13}C$ which was unrelated to light environment and bulb width, explaining the weaker, but nevertheless significant, correlation between $\delta^{13}C$ and bulb width compared to the correlation reported between chl a/b ratio and bulb width (Fig. 2.6 b,d).

The relationship between soil moisture and the wild leek phenology index was unexpected, but could be explained by a 23-day period from 17 April to 9 May 2013. Only 9.6 mm and 19 mm of rain fell respectively in the Lower Laurentides and Estrie during this period (Environment Canada, 2013), at the end of which plants from some plots started to show early signs of senescence. Similarly, Tessier (2008) reported lower leaf longevity for *Trillium erectum* during a dry summer.

2.6.4 Litter

The negative relationship that was observed between litter cover and total light was expected, since a denser canopy should shed more leaves (Lebret et al., 2001). Although the chl a/b ratio and $\delta^{13}C$ analyses confirmed that the direct effect of canopy type on wild leek individual growth mostly resulted from differences in light availability, there could still be an important effect of litter on wild leek through its impact on soil temperature. The present results show that litter reduced soil temperature variance and average. This is in agreement with the effects described by Facelli & Pickett (1991), i.e., that litter is known to stabilize soil temperature, to isolate the soil from the air, and to intercept solar radiation. This resulted in greater wild leek survival in plots with a higher litter cover. Baltzinger et al. (2012) also reported greater leafing success for the spring ephemeral *Anemone nemorosa* L. when litter was present. They also reported greater rhizome growth in plots with litter, whereas we found that wild leek growth increased with increased soil temperatures, as already discussed. Higher soil temperatures in early spring would lengthen the growing

season, thereby benefiting wild leek, but higher soil temperatures later in the season might incur mortality.

2.6.5   Nutrient availability

We expected that the most important ratios influencing wild leek growth would involve macro-nutrients, but the fact that the [Fe,Mn | Zn,Cu] ratio emerged as the ratio most strongly influencing wild leek growth suggests that the fertilization regime that had been established by Bernatchez et al. (2013) and applied in the present experiment did provide well-balanced macronutrient availability. The observed negative relationship between [Fe,Mn | Zn,Cu] balance and bulb width and seed production could result from either Mn and Fe toxicity or Zn and Cu deficiency. When compared to general reference values (Broadley et al., 2012) and those reported for leek (*Allium porrum*, currently classified as *A. ampeloprasum* L.) shoots (Sorensen et al., 2005), leaf Cu content of ramp or wild leek appeared to be quite low, whereas Zn values are in the normal range and Mn and Fe concentrations are far below toxic levels. The organic fertilizers that were used contained a small amount of Mn and Fe, but no Cu or Zn, and could have contributed to the observed imbalance. Cu is important for oxidation-reduction reactions, e.g., photosynthesis, and plays a role in cell wall lignification. Its deficiency is known to affect fruit and seed production, because Cu-deficient pollen is usually sterile (Broadley et al., 2012).

Wild leek leaves are rich in Ca and Mg (Nault & Gagnon, 1988; Davis & Greenfield, 2002; Ritchey & Schumann, 2005), which could explain the positive relationship that was observed between total leaf width and [Mg,Ca,K | S,P,N] balance. Leaf Ca was in the same range as has been reported previously for wild leek (Bernatchez et al., 2013). Furthermore, the fertilizers contained P, N, Ca, K and S (sulfate), but only a small amount of Mg; thus, it is possible that some plots were slightly Mg-deficient, explaining the positive relationship between total leaf width and the [Mg,Ca,K | S,P,N] balance.

Soil pH has a major impact on nutrient availability (White, 2012), which is reflected by the strong relationships that were observed with most of the mineral balances (Table 2.2). It is surprising that soil pH did not explain variation in any of the wild leek growth

variables, considering that nutrient balances are linked so strongly to soil acidity and that soil pH ranged from 4.09 to 6.72. Wild leek is known to thrive in acid soils with pH values as low as 4.9 (Davis & Greenfield, 2002), while lime application in soil with pH 4.0 improves wild leek growth (Ritchey & Schumann, 2005). Acidity could be less important compared to light or litter cover, when plants are fertilized to compensate for potential reductions in nutrient availability and when Ca is added to counteract other negative effects of low pH, such as Al toxicity, as has already been reported for American ginseng (Nadeau et al., 2003).

2.6.6 Light in natural stands

Light did not appear to be an important factor explaining population growth in natural populations, although higher light availability did provide slightly better seed production. The marginal effect of light contradicts the stronger relationship that was observed in the transplant plots. Light conditions were recorded only for the 2013 growing season, while population growth data covered 11 years, from 2003 to 2013. Canopy structure could have varied significantly in this period through gap formation and closure (e.g. Diaci et al., 2012). Nevertheless, wild leek phenology acclimated to the tree canopy phenology in wild populations as well as in the transplant experiment. This response confirms the plasticity of wild leek phenology in response to its light environment. The absence of a relationship between canopy closure index and annual population growth was unexpected, despite a positive relationship between wild leek phenology index and annual population growth. Other environmental factors could have influenced wild leek phenology and population annual growth, such as soil moisture or temperature, as discussed previously.

2.6.7 Concluding remarks

In the last few decades, overharvesting caused a severe decrease in wild leek abundance in southern Québec (Dagenais, 1985; Couillard, 1995). This led to its protection by provincial laws, which now strongly restrict harvesting. Recent studies have shown that wild leek is a good candidate for forest farming (Davis & Greenfield, 2002; Facemire, 2008), which could reduce harvesting pressures that have been imposed on natural stands.

A longer period before spring canopy closure, combined with a dense canopy during summer, significantly improved wild leek growth in cultivated forest plots. More specific research is still needed to differentiate the influence of various tree species so that interested producers can be properly advised regarding optimal locations for growing wild leek. We can already state that late bud-bursting genera, such as *Fraxinus*, *Juglans*, *Quercus* and *Tilia*, provide an optimal light environment for spring ephemerals. Specific canopy composition could be less important in natural populations, where other environmental factors, such as soil moisture, nutrient availability or temperature can vary greatly among populations. Further research is required to assess the importance of litter composition and abundance, soil moisture, and micro-nutrient availability for wild leek growth. Nevertheless, this study confirms the plasticity of wild leek leaf phenology, highlights its capacity to acclimate to changes in light availability, and emphasizes the importance of canopy phenology and total light availability for growth and reproduction of spring ephemerals.

2.7 Acknowledgments

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Chapitre 3.   Sustainable leaf harvesting and impact of plant density in wild leek cultivation plots and natural stands of Southern Quebec, Canada.

3.1   Abstract

Wild leek (*Allium tricoccum*) is an edible forest geophyte popular in northeastern America. In the last decades, overharvesting caused a major decline in its natural populations of southern Québec, Canada. Sustainable exploitation could be achieved through forest farming.

Natural wild leek stands can reach high densities of ca. 400 bulbs m^{-2}, which could reduce yield due to competition. Previous studies recommended very low levels of sustainable bulb harvest, but did not consider the potential improved growth following a reduction in density. Limiting the harvest to leaves only may also provide an alternative form of exploitation, but could slow growth by reducing both carbon and nutrient reserves. Our objectives were to assess the effects of (1) planting density and post-harvest density reduction and (2) the timing and intensity of leaf harvest on subsequent growth and reproduction of wild leek.

Three experiments were set up. Bulbs were planted at various densities covering the range surveyed in natural populations. Plots set up in dense populations were subjected to different harvesting intensities. Cultivated plots were subjected to different percentages of leaf harvesting, at different times during the growing season.

Plants growing in higher density plots exhibited slower growth and reproduction rates, but a higher productivity per cultivated area. A similar effect, albeit only marginal, was obtained following bulb harvests in natural populations. Harvesting leaves did not affect survival, but delaying the harvest and harvesting only half of the leaves favored subsequent plant growth. Those results will help improve wild leek yields in forest farming.

Keywords : *Allium tricoccum*; Bulb harvest; Planting density; Forest farming; Leaf harvest

3.2 Résumé

L'ail des bois (*Allium tricoccum* Ait.) est une herbacée forestière pérenne comestible populaire dans le nord-est de l'Amérique. Au sud du Québec (Canada), la récolte abusive a causé la disparition de plusieurs populations naturelles. La culture sous couvert boisé pourrait en permettre une exploitation durable.

Les populations naturelles peuvent atteindre une densité de 400 bulbes m^{-2}, ce qui pourrait ralentir la croissance à cause de la compétition. Les taux de récolte suggérés par les recherches précédentes sont très faibles, mais ne tiennent pas compte de la hausse potentielle du taux de croissance suivant une baisse de densité. Récolter uniquement les feuilles pourrait aussi être un mode d'exploitation durable, mais ralentirait la croissance subséquence en réduisant les réserves de carbone et de nutriments. Nos objectifs étaient de déterminer l'effet (1) de la densité de plantation et la réduction de densité post-récolte et (2) du moment et du pourcentage de récolte des feuilles sur la croissance et la reproduction subséquentes de l'ail des bois.

Trois expériences ont été mises en place. Des bulbes ont été plantés à différentes densités représentatives de celles observées en populations naturelles. Des parcelles en populations denses ont été soumises à différentes intensités de récolte. Des parcelles cultivées ont été soumises à différents pourcentages de récolte de feuilles à divers moments dans la période de croissance.

Les parcelles à forte densité ont eu une croissance et reproduction plus lente, mais un meilleur rendement par surface cultivée. Un effet similaire, quoique marginal, a été observé suite à la récolte de bulbes en populations naturelles. Récolter les feuilles n'a pas affecté la survie, mais retarder la récolte plus tard en saison et récolter seulement la moitié du feuillage ont favorisé la croissance subséquente. Ces résultats permettront d'optimiser les rendements de la culture en boisé de l'ail des bois.

Mots-clés

Allium tricoccum; Récolte de bulbes; Récolte de feuilles; Densité; Culture en boisé

3.3 INTRODUCTION

Wild leek (*Allium tricoccum* Ait.) is a spring ephemeral geophyte very popular in northeastern America for the taste of its bulb. Its one to three leaves unfold early in spring right after snowmelt (late April in Quebec) and senesce a few weeks later, following canopy closure (late May). Anthesis occurs in July and seeds are mature in late August (Jones, 1979). It can take 7-10 years from seeds to reach plant maturity (Nantel et al., 1996), and most reproductive effort is through asexual reproduction by division of the bulb (Nault & Gagnon, 1993). In the last decades, overharvesting caused an important decline in its natural populations in southern Quebec, Canada (Dagenais, 1985; Couillard, 1995), which led to its protection by provincial law as an endangered species. Commercial harvesting is now forbidden, but illegal harvesting continues. Recent studies have shown that this species is a good candidate for forest farming (Davis & Greenfield, 2002; Facemire, 2008), which could help reduce pressure on natural populations.

The average density recorded in natural wild leek stands is around 90 bulbs m^{-2} (Dagenais, 1985; Nault & Gagnon, 1993), but dense patches can reach as high as 350-400 bulbs m^{-2} (personal observation). In a forest farming context, such densities could affect growth by introducing competition among individuals. Nault & Gagnon (1993) already reported annual mortality in the center of dense wild leek patches due to overcrowding. Crowding effect is well studied on a wide density range in the commercial onion, *Allium cepa* L: McGeary (1985) reported a decrease in bulb diameter from 4.7 to 2.1 cm and in dry weight from 12.3 to 1.0 g bulb^{-1} if density was increased from 178 to 1600 bulbs m^{-2}; bulb fresh weight decreased from 135 to 71 g when density increased from 43 to 117 bulbs m^{-2} (Brewster & Salter, 1980); increasing density from 397 to 484 bulbs m^{-2} reduced bulb fresh weight from 276 to 244 and from 232 to 201 g bulb^{-1} in an experiment repeated two consecutive years (Leskovar et al., 2012); onion seeds sown and transplanted at a density of 26 bulbs m^{-2} produced less mature bulbs in the bulb diameter class of 10.2-12.7 cm than at a density of 9 bulbs m^{-2} (Herison et al., 1993). Previous trials on wild leek planted at a density of 11 and 44 bulbs m^{-2} indicated no effect of density on bulb dry weight, which was respectively 0.27 and 0.25 g bulb^{-1} (Ritchey & Schumann, 2005). These densities might however be too low to induce competition.

The maximum harvest rate applicable to natural populations without potential long term decline in number was modelled and estimated at less than 10% annually by Nault & Gagnon (1993), 8% by Nantel et al. (1996), and at as little as 10% per 10 years by Rock et al. (2004). However, these studies did not consider the impact of bulb density on subsequent growth in their models. An increase in annual bulb growth rate following release of plant competition would not only favour bulb size, but also bulb division and seed production as larger bulbs are more likely to flower or divide (Nault & Gagnon, 1993). Furthermore, seedling survival could be improved since they would have access to more resources.

Although wild leek is mainly sold as bulbs, leaves are also edible and their harvest is usually considered as a more sustainable exploitation of wild leek, knowing that it does not automatically kill the plant (Dagenais, 1985). Nevertheless, leaves are a major investment in carbon and nutrients. After full unfolding, they usually account for 50% of total biomass and contain more than 60% of P, K, and Mg, and up to 90% of total N and Ca (Nault & Gagnon, 1988). Wild leek is however very efficient in translocating nutrients to the bulb during leaf senescence. Even Ca, which cannot be translocated, appears to be reabsorbed by the roots following leaf decomposition, as roots remain active after leaf senescence (Nault & Gagnon, 1988; Rothstein & Zak, 2001b). Leaf harvest can also affect the annual carbon budget of the plant, as green sections of partially senesced leaves continue to photosynthesize (Bernatchez & Lapointe, 2012). Leaf harvest could therefore be deleterious to wild leek by loss of nutrients and carbon fixation capacity. Indeed, previous studies on other *Allium* species assess the negative effect of defoliation. *A. sativum* L. (garlic), *A. ampeloprasum* L. ssp. *porrum* (leek) and *A. cepa* L. all produce smaller bulbs and their yield is lower following defoliation (Muro et al., 1998; Muro et al., 2000; Irigoyen et al., 2010). This effect is highest in *Allium* species when all leaves are just completing unfolding, at the beginning of the bulb formation (Irigoyen et al., 2010). Early defoliation also significantly affects subsequent growth in other spring flowering species such as *Trillium erectum* L., *Clintonia borealis* (Raf.) Ait., *Claytonia virginica* L. and *Maianthemum canadense* Desf. (Whigham & Chapa, 1999; Lapointe et al., 2010). Partial harvest of the foliage could also be less deleterious than total harvest by allowing the remaining leaves to continue photosynthesis and to translocate their nutrients to the bulb

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during leaf senescence. Moreover, partial defoliation may induce compensatory photosynthesis on the remaining leaves (Nowak & Caldwell, 1984; Meyer, 1998; Maurin & DesRochers, 2013).

The first objective of the present study was to determine if high densities, close to those recorded in some natural stands, impede wild leek growth under cultivation. Four densities were thus compared: 44, 88, 178 and 356 bulbs m^{-2}. The second objective was to assess the effect of a reduction in density following partial bulb harvest on the subsequent growth of wild leek in natural populations. For these first two objectives, we posit that competition among individuals rises with increasing density, resulting in both reduced individual plant growth, and reduced population growth. The third objective was to evaluate the impact of different levels of leaf harvest, taking place at different times during the growth season, on survival and growth of wild leek. We posit that harvesting a higher percentage of leaf area early in the season will negatively affect wild leek by reducing the total amount of carbon fixed and by preventing the recycling of nutrients invested in the leaf.

3.4 Material and methods

3.4.1 Study sites

All experiments were conducted in the Basses-Laurentides (Lower Laurentian) region, Southern Quebec, Canada, except for two sites of the Bulb Harvesting experiment, which were located in Estrie (Eastern Townships), also in Southern Quebec. The climatic conditions in Lower Laurentides are: mean annual temperature 5.0°C, 1065 mm of precipitations, and 1866 degree-days over 5°C annually. Estrie has a mean temperature of 4.1°C, precipitations of 1144 mm and 1637 degree-days (Environment Canada, 2013). Those regions are close to the northern limit of wild leek distribution (Jones, 1979; Dagenais, 1985), but encompass several large natural populations of wild leek (pers. obs.). Exact locations of the sites will not be disclosed in order to prevent unauthorized harvesting.

3.4.2 Planting density

Two randomized complete blocks were set up on two different sites in spring 2008. Each block contained 12 plots, i.e. three replicates of each of the four densities tested (44, 88, 178, 356 bulbs m^{-2}. Each plot consisted of 100 bulbs planted 10 per row in 10 rows in the appropriate plot size to reach the target density. The bulbs came from a seizure by governmental authorities of illegally harvested bulbs. All transplanted bulbs were in good condition, and of the *tricoccum* variety (Jones, 1979).

Plots were plowed 15 cm deep, then fertilized with 12-25-19 kg ha^{-1} of N-P$_2$O$_5$-K$_2$O (Bio-Jardin 4–3–6 [N–P$_2$O$_5$–K$_2$O], which also contains 3 % Mg, mixed with Fossil Bone 0–13–0, McInnes Natural Fertilizers inc., Stanstead, QC, Canada) and 1350 kg ha^{-1} of gypsum (Uncalcined Gypsum Products, CaSO$_4$, Georgia-Pacific Gypsum Corporation, Atlanta, GA, USA) in spring 2008, then with 15-30-23 kg ha^{-1} of N-P$_2$O$_5$-K$_2$O and 1650 kg ha^{-1} gypsum in 2009. From 2010 to 2013, plots were fertilized at a higher level, 27-55-42 kg ha^{-1} of N-P$_2$O$_5$-K$_2$O and 3000 kg ha^{-1} of gypsum as proposed by Bernatchez et al. (2013).

From 2009 to 2012, Total Leaf Width was measured with a ruler on each plant (TLW$_i$) of three randomly chosen rows in each plot after complete leaf unfolding. TLW$_i$ is the sum of the width of all leaves carried by an individual and measured at their largest point. Individual Bulb Width (BW$_i$) was measured with a caliper on four rows per plot in July 2012 only, soon after leaf senescence, that is when leaf carbon and nutrients translocation to the bulb had been completed (Nault & Gagnon, 1988). Bulb survival was noted in 2009 and 2010. From 2011 to 2013, the total number of bulbs per plot was counted instead of survival, since crowding in denser plots rendered difficult the distinction between divisions of a same mother bulb and two independent bulbs. The number of floral scapes was noted in 2011, which was a good flowering year.

Two annual measures of bulb yield were calculated: (1) by estimating the annual increase in bulb fresh mass in grams yr^{-1} per bulb initially planted, and (2) by expressing it in grams yr^{-1} m^{-2}. The mean individual bulb fresh mass (FMB$_i$) in grams was estimated using the equation,

$$FMB_i = 0.250 \times TLW_i - 0.254 \qquad \text{Eq. 3.1}$$

($r^2 = 0.728$; $P < 0.001$; $n = 214$; 2.6 cm < TLW_i < 23.3 cm). The FMB_i data were then summed for the whole plot, then either divided by the number of bulbs initially planted or by the surface area of the plot. Annual increment in biomass was then calculated for each plot.

In addition to the individual bulb growth, it is important to consider the total growth of all daughter bulbs issued from the divisions of a same mother bulb. However, since the simple addition of the BW_i would inflate the effect of bulb division, the sum of daughter bulbs width issued from a same mother bulb (BW_d) was calculated by adding up the transversal bulb areas at their largest point, calculated from their diameter, then by extracting the diameter of this larger virtual bulb. It resulted in the equation,

$$BW_d = 2 \times \sqrt{\frac{\sum_{i=1}^{n} \pi r_i^2}{\pi}} \qquad \text{Eq. 3.2}$$

where n is the number of daughter bulbs and r_i is the radius of the ith daughter bulb. The TLW for all daughter bulbs issued from a same mother bulb (TLW_d) was however a direct addition of all leaf widths. TLW_d and BW_d provide insight on the overall production of each initial bulb, even after some of them have divided.

In 2012, there was an outbreak of a parasite, which was identified as *Blaniulus guttulatus* Fabricius, a millipede common in North America and introduced from Europe. It is a known root parasite in agriculture (Hopkin & Read, 1992; Fraval, 2014), but we did not find any report of it attacking *Allium* species. Hence, TLW and scape production from 2012 and onward were excluded from the statistical analyses. BW, which was only measured in 2012, included only plot sections which were not yet affected by *B. guttulatus*. The number of bulbs per plot was noted in 2012 and 2013 in order to determine if survival was better in less dense plots.

3.4.3 Bulb harvesting

The usual method for harvesting bulbs is of great concern in wild leek natural populations. As natural populations can reach very high densities, non-targeted bulbs can be damaged while digging for specific plants. It is important to harvest individual bulbs rather than bunches of bulbs to help population recovery (Nault & Gagnon, 1993). However, this is

virtually impossible to do in dense stands. The selected harvesting method consisted in digging out all of the bulbs in a small quadrat, hand-picking the ones to harvest, and replanting the others within the same area. Harvested bulbs were of class 4 and 5, *i.e.* medium-sized plants of 3.8 cm < TLW ≤ 10 cm (Nault & Gagnon, 1993). We thus replanted those too small for commercial harvest along with the large reproducing bulbs, to promote regeneration of the plot.

In four natural wild leek high-density populations (two in Lower Laurentides and two in Estrie), 12 plots were initially delimited, containing ca. 100 bulbs each, for a total of 48 plots. Four different harvesting treatments were randomly assigned to the plots in spring 2011, before leaf senescence. Two treatments consisted in harvesting 20 and 40 % of the bulbs (which were replanted nearby). The other treatments were two different controls. In the first one, which will be referred to as "0 %", all the bulbs were dug out and replanted; in the second one, referred to as "Control", the bulbs were left untouched. These two controls were necessary to test the effect of the harvesting method itself.

During springs 2012 to 2014, TLW_i of all individuals was measured after they had completely unfolded. The number of floral scapes and number of seeds produced per plot were recorded in 2013, which was a good flowering year. The juvenile ($TLW_i < 1$ cm) and non-juvenile bulbs were counted in order to assess population growth after the treatments. The size of the plots was also measured and used as a covariable in the statistical analysis, as it influences plant density.

3.4.4 Leaf harvesting

The leaf harvesting experiment was conducted in plots set up in 2008 (see Bernatchez et al., 2013, for details). Each 90×315 cm plot consisted of 100 bulbs planted in five rows of 20 plants. There were six plots in each of four sites, for a total of 24 plots. Each plot had received a combination of different N-P-K fertilizer and/or gypsum doses in spring 2008 and 2010. By autumn 2010, plots no longer differed in terms of soil extractable P, Ca^{2+} and Mg^{2+} (Bernatchez et al., 2013). From 2011 to 2013, fertilizers were applied annually in each plot in early spring, at the same doses as in the planting density experiment for the same years.

During spring 2011, 36 bulbs per plot were randomly selected among those which had not divided yet. We selected plants with the same number of leaves (2 or 3) within a plot whenever possible. We applied to each selected bulb a combination of the following treatments: 0, 1 (50% leaf area) or 2 (100% leaf area) leaves harvested, 15, 20 or 25 days after full leaf unfolding, for a total of nine combinations, replicated four times per plot. In the cases where a bulb had three leaves and was selected for the one-leaf harvest, we removed one leaf and the distal half of a second one, in order to remove 50% of the total leaf area.

TLW_d and BW_d were measured from 2011 to 2013. Floral scape emergence was noted each year, then scapes were immediately removed in order to limit the expense in energy devoted to sexual reproduction and to reduce variance among individuals. Bulb division was also recorded.

3.4.5 Statistical analysis

Statistical analysis for the planting density and the leaf harvesting experiments were performed with the software *SAS* 9.3 (SAS institute Inc., Cary, NC, USA).

For the planting density experiment, mixed effects ANOVAs were performed, using the block as random variable, on all response variables within each year they were measured. Multiple comparisons were performed using the Tukey HSD test. From Yr 2 to Yr 4 (2009 to 2011), the analysis was also performed as repeated measures of the TLW_i and TLW_d to determine if there was a planting density × year interaction.

For the bulb harvesting experiment, mixed effects ANCOVAs within each year from Yr 2 to Yr 4 (2012 to 2014) were performed for the following variables: TLW of the whole plot (TLW_{plot}; obtained by adding up the widths of all leaves in the plot), mean TLW_i, number of bulbs and number of juveniles per plot. The site was considered as a random variable. ANCOVAs were also performed on relative growth from Yr 2 to 4 and on scape and seed production in Yr 3. We used plot size (in cm^2) as a covariable in order to compensate for the variability in pre-treatment density (see next paragraph for further details). The Tukey HSD test was used for multiple comparisons among the four treatments.

Despite the fact that treatments were randomly assigned among the pre-delimited plots, we noticed that the resulting differences in density were smaller than expected. Indeed, post-treatment density was not significantly different between the 40%, 20% and 0% treatments, although the 40% plots were effectively sparser than the C plots (Figure 3.1; $F_{3, 21} = 3.36$, $P = 0.038$). There was also a huge variance in density within a treatment, especially among the control plots, in which it varied from 151 to 634 bulbs m^{-2}. This kind of density variation can influence the growth of wild leek (see results from the planting density experiment). To more accurately assess the effect of a reduction in bulb density following a partial harvest in natural populations, we decided to conduct a second series of analyses where: (1) we left aside the experimental treatments and used the density (bulbs m^{-2}) recorded immediately after harvest treatments as an independent variable; and (2) we excluded the "control" plots from the analysis, since the first analyses indicated that this group differed from the 0% plots (see Results and Discussion for more details). We then performed correlation tests between the different response variables and the post-treatment density recorded in Yr 1 (2011), using the software R 2.15.1 (*R Core team*, 2012).

In the leaf harvesting experiment, two plots were pillaged in spring of Yr 1 on one site. They were therefore removed from the analysis. Two other plots were also pillaged in spring of Yr 2 on the same site as the first two, after leaf width and before bulb width measurements, and were not considered in the analysis of subsequent measures.
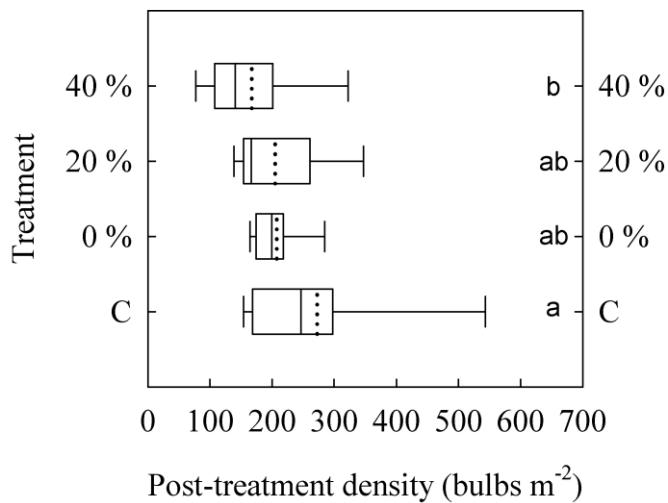
Figure 3.1 Post-treatment density immediately after the different bulb harvest treatments have been applied (Yr 1). Dotted line indicates the mean, the continuous line, the median. Treatments with a same letter are not significantly different according to the Tukey HSD test ($F_{3,\,21}$ = 3.36; P = 0.038).

The experimental design contained 3 sub-groups with no leaf harvesting, one for each date of leaf harvesting. As explained by Gates (1991), we cannot analyze these data as a 3 × 3 factorial experiment, because it would artificially inflate the interaction between the two factors. A correct way of analysing such experimental design was suggested by Lynch et al. (2008). As a first step, the interaction between the two factors was analyzed in a 3 × 2 factorial analysis ('number of days before harvest' × 'percentage leaf area'), excluding the plants which had no leaf harvested. Mixed effects ANOVAs were performed on BW_d and TLW_d with repeated measures: Yr 2 and Yr 3 for the TLW_d and Yr 1 to Yr 3 for the BW_d. Leaf measurements of Yr 1 were not included, because they were already unfolded at the time the treatment was applied. Site, plot and year were considered as random variables in the linear mixed model. In a second step, all plants which had no leaf harvested were pooled together and considered as a 'control' treatment. ANOVAs and multiple comparisons among all seven treatment combinations including the controls were performed within each year, using the Tukey HSD test, for TLW_d (Yr 2 and Yr 3), BW_d (Yr 1 to Yr 3), floral scapes (Yr 3, which was a good flowering season), bulb division (Yr 2 and Yr 3) and survival after two years (Yr 3). For bulb division, flowering and survival, a

logistic function was used to model the probability that the binary events "division", "flowering" and "survival" occur.

3.5 Results

3.5.1 Planting density

Planting density significantly influenced most of the growth parameters recorded (Table 3.1). Only the last year data are presented (Yr 4 or 5), except for survival where Yr 2 and Yr 3 results confirmed that there was no transplant shock, and survival ranged from 87.8% to 95.5%. Three years after transplantation (Yr 4), wild leek had higher TLW_i and TLW_d in less dense plots (Table 3.1 and Figure 3.2). Vegetative reproduction was also more frequent in sparsely planted plots: the number of bulbs per plot in Yr 4 had increased by 47.3% in the lowest density plots (44 bulbs m^{-2}), by 32.7% in the 88 bulbs m^{-2} plots and had remained at levels similar to the original planting numbers in the two highest densities (Figure 3.3). This led to a significant interaction between TLW_d and Yr (Figure 3.2b): plant growth and bulb division were both higher in plots with low density, whereas the density×year interaction was not significant for TLW_i (Figure 3.2a).

In accordance with the TLW_i and TLW_d data, bulb width, measured in Yr 5, was also higher in low-density plots, both when reported individually (BW_i) and as a sum of all bulb width originating from the same mother bulb (BW_d; Table 3.1). As larger bulbs tend to flower more frequently, the 44 bulbs m^{-2} plots produced more scapes in Yr 4 than the 356 bulbs m^{-2} plots. The annual yield from Yr 2 to Yr 4 in grams of fresh mass of bulb per year per bulb planted was higher in sparser plots. The annual yield in terms of grams of fresh mass of bulb per m^2 per year however followed the opposite trend, and was higher in the 356 bulbs m^{-2} plots than in the 44 and 88 bulbs m^{-2} plots.

There was no significant difference between the two lowest densities (44 and 88 bulbs m^{-2}), for any of the variables (Table 3.1). At a density of 178 bulbs m^{-2} and up, BW_d, TLW_d, the number of bulbs per plot and the annual bulb yield (g FM yr^{-1} per bulb initially planted) significantly decreased compared to the lowest density (44 bulbs m^{-2}). Only the highest density (356 bulbs m^{-2}) significantly decreased BW_i, TLW_i and flowering measured the last year before the parasite outbreak, as compared with the lowest density.

Table 3.1 Analysis of variance and multiple comparisons of wild leek growth responses among different planting densities.

Variable	Density (bulb m^{-2})[†]				std. err.	F-val	P-val
	44	88	178	356			
Annual bulb yield[‡] (g FM yr^{-1} m^{-2})	56.1 a	86.5 a	108.5 ab	165.5 b	20.8	6.36	0.005
Annual bulb yield[‡] (g FM yr^{-1} per bulb planted)	1.26 a	0.97 ab	0.61 bc	0.47c	0.14	9.77	< 0.001
BW$_i$ Yr 5 (cm)	1.62 a	1.64 a	1.50 ab	1.33 b	0.05	8.05	0.003
BW$_d$ Yr 5 (cm)	2.47 a	2.21 ab	2.02 bc	1.79 c	0.13	11.67	< 0.001
TLW$_i$ Yr 4 (cm)	10.02 a	9.22 a	9.03 a	7.36 b	0.72	10.22	< 0.001
TLW$_d$ Yr 4 (cm)	15.50 a	13.71 ab	11.46 bc	9.81 c	1.34	19.93	< 0.001
Survival Yr 2 (%)	95.5	95.5	93.8	96.0	1.18	1.28	0.318
Survival Yr 3 (%)	93.0	94.2	89.2	87.8	2.58	1.86	0.180
Bulb number Yr 4[*]	147.3 a	132.7 ab	99.7 b	99.0 b	13.3	3.88	0.031
Scape per bulb Yr 4	0.325 a	0.193 ab	0.247 ab	0.168 b	0.05	4.53	0.019

[†] Within a line, values followed by different letters were significantly different according to the Tukey-Kramer test ($\alpha = 0.05$).
[‡] Annual yields represent the mean annual increment from Yr 2 to Yr 4. Planting took place in Yr 1.
[*]100 bulbs were initially planted in each plot.
Abbreviations: BW$_i$, individual bulb width; BW$_d$, sum of daughter bulbs width issued from a same mother bulb ; TLW$_i$, individual total leaf width; TLW$_d$, total leaf width of all daughter bulbs issued from a same mother bulb; FM, fresh mass.
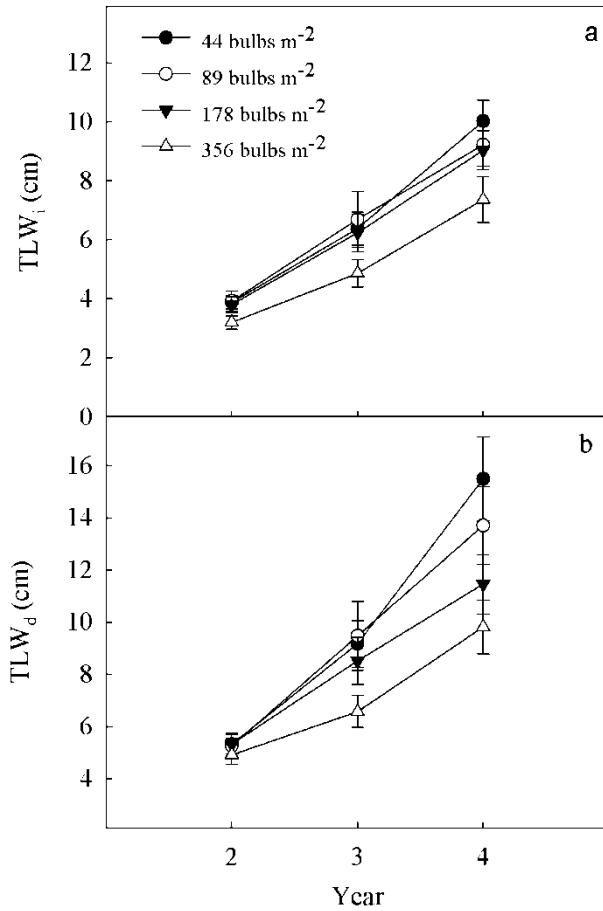
Figure 3.2 Interaction between planting density effect and year for individual total leaf width (TLW$_i$) and TLW for all daughter bulbs from a same mother bulb (TLW$_d$). Mean $\pm$ SE. Results of ANOVA for TLW$_i$: Density effect (D) ($F_{3,55}$ = 12.2, $\textbf{\textit{P}}$ < $\textbf{0.001}$), Year effect (Yr) ($F_{2,\,55}$ = 203.9, $\textbf{\textit{P}}$ < $\textbf{0.001}$), D $\times$ Yr ($F_{6,\,55}$ = 1.46, P = 0.21). ANOVA for TLW$_d$: D ($F_{3,\,55}$ = 12.2, $\textbf{\textit{P}}$ < $\textbf{0.001}$), Yr ($F_{2.\,55}$ = 136.8, $\textbf{\textit{P}}$ < $\textbf{0.001}$), D $\times$ Yr ($F_{6,\,55}$ = 3.77, $\textbf{\textit{P}}$ = $\textbf{0.003}$). Planting took place in Yr 1.

Figure 3.3 presents the reduction in bulb number probably caused by the parasite outbreak in Yr 5 and 6 compared with the numbers recorded in Yr 4. The effects of density and the variation from one season to the other were significant. The less dense plots seemed initially less affected by the parasite from Yr 4 to 5, but once their numbers began to drop, they did so at a similar rate as denser plots, and the interaction between density and year was not significant.
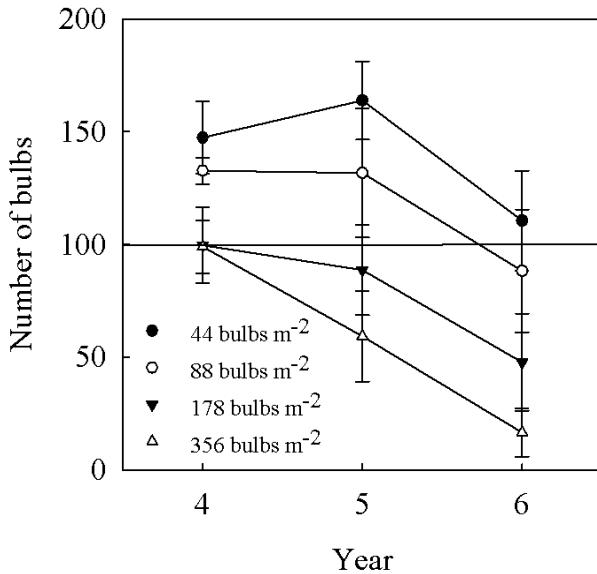
Figure 3.3 Number of bulbs (mean ± SE) per plot from Yr 4 to Yr 6 as a function of planting density. The horizontal line indicates the number of bulbs planted per plot (100). Results of ANOVA: density effect (D) ($F_{3,\ 54}$ =14.1, $P < 0.001$); year effect (Yr) ($F_{2,\ 54}$ = 11.7, $P < 0.001$); D × Yr effect ($F_{6,\ 54}$ =0.54, $P = 0.774$). Planting took place in Yr 1.

3.5.2   Bulb harvesting

One site exhibited surprisingly low survival of the replanted bulbs, probably because of the high temperature prevailing on the date of transplantation. In 2013, only 40, 20 and 13 bulbs were still alive on average in the 0%, 20% and 40% plots, respectively, while 90 bulbs survived in the control plots. This site was therefore discarded from further statistical analysis. Three plots from another site – two "0%" and one "20%" – were also abandoned for the same reason.

Relative increase of TLW_{plot} and number of bulbs per plot did not differ among treatments from Yr 2 to Yr 4, according to the ANCOVA (Table 3.2). Yet, relative increase of TLW_i tended to be higher in treated plots ($P = 0.094$) suggesting a positive effect of the digging / replanting treatment on relative growth of individual plant (TLW_i). It is worth mentioning that TLW_i increased in all groups between Yr 2 (2012) and Yr 3 (2013), then decreased between Yr 3 (2013) and Yr 4 (2014). The average number of scapes and seeds produced per bulb and the number of juveniles per plot did not differ among treatments. In Yr 2, TLW_i was 28% higher in control plots than in the three harvesting treatments, indicating a negative effect of the harvesting method. This difference was less pronounced

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Table 3.2 Effects of different percentages of bulb harvesting on growth response in natural stands of wild leek, during the three subsequent years. Data are presented as the expected values at mean plot area as modeled by the ANCOVA.

Variable	Year(s)	Treatment				$F_{3,20}$	P-val
		C	0 %	20 %	40 %		
Rel. increase of TLW_{plot}[†]	Yr 4 / Yr 2	-0.125 (0.104)	0.134 (0.117)	0.034 (0.111)	0.044 (0.104)	0.72	0.550
Rel. increase of TLW_i[*]	Yr 4 / Yr 2	-0.095 (0.064)	0.093 (0.071)	0.083 (0.068)	0.046 (0.064)	2.44	*0.094*
Rel. increase of bulb number[‡]	Yr 4 / Yr 2	-0.031 (0.074)	0.038 (0.085)	-0.105 (0.079)	0.021 (0.074)	0.62	0.613
Scape per bulb	Yr 3	0.214 (0.031)	0.097 (0.036)	0.158 (0.033)	0.141 (0.031)	2.12	0.130
Seeds per bulb	Yr 3	1.859 (0.344)	0.768 (0.393)	1.462 (0.365)	1.382 (0.343)	1.77	0.185
TLW_{plot}	Yr 2	804.5 (39.4) a	635.5 (43.9) b	506.1 (41.6) c	446.6 (39.2) c	22.50	**< 0.001**
(cm)	Yr 3	893.0 (45.3) a	795.4 (51.8) ab	674.9 (48.1) b	532.6 (45.2) c	11.62	**< 0.001**
	Yr 4	701.7 (59.0) a	715.2 (65.7) a	510.3 (62.4) b	461.37 (58.8) b	6.44	**0.003**
TLW_i	Yr 2	7.68 (0.33) a	5.80 (0.36) b	6.10 (0.35) b	6.11 (0.33) b	12.22	**< 0.001**
(cm)	Yr 3	8.81 (0.45) a	7.44 (0.49) c	8.57 (0.47) ab	7.71 (0.45) bc	3.37	**0.039**
	Yr 4	6.87 (0.27)	6.26 (0.30)	6.30 (0.28)	6.33 (0.27)	1.54	0.235
Juveniles per plot (TLW_i < 1 cm)	Yr 2	29.94 (9.87)	19.97 (11.26)	39.57 (10.48)	26.20 (9.84)	0.59	0.628
	Yr 3	22.17 (7.27)	33.90 (8.11)	28.12 (7.69)	37.68 (7.25)	1.17	0.345
	Yr 4	23.83 (8.27)	30.58 (8.85)	23.63 (8.60)	25.72 (8.25)	0.30	0.823
Bulbs per plot (TLW_i > 1 cm)**	Yr 2	104.8 (4.2) a	109.2 (4.8) a	84.2 (4.4) b	73.0 (4.2) b	15.59	**< 0.001**
	Yr 3	102.2 (5.2) a	109.7 (6.0) a	80.1 (5.5) b	69.3 (5.2) b	11.83	**< 0.001**
	Yr 4	101.5 (7.8) a	114.2 (8.9) a	78.2 (8.3) b	73.2 (7.8) b	5.66	**0.006**

Notes:

ANCOVA performed with plot area as covariable. Value in parenthesis is the standard error. Within one line, values followed by the same letter are not significantly different according to Tukey HSD test (α = 0.05).

[†] Relative increase of the sum of TLW_i for the whole plot (TLW_{plot}): ($TLW_{plot\_Yr 4} - TLW_{plot\_Yr 2}$) / $TLW_{plot\_Yr 2}$

[*] Relative increase of the TLW_i: ($TLW_{i\_Yr 4} - TLW_{i\_Yr 2}$) / $TLW_{i\_Yr 2}$

[‡] Relative increase of the number of bulbs: (Nb Bulbs$_{Yr 4}$ − Nb Bulbs$_{Yr 2}$) / Nb Bulbs$_{Yr 2}$

**100 bulbs were initially planted in each plot.

Abbreviations: TLW_i, mean individual total leaf width ; TLW_{plot}, total leaf width of all plants with a TLW_i > 1 cm inside a plot.

in Yr 3, as the 20% plots were no longer significantly different from the control, but TLW_i of the 0% and 40% plots remained lower. There was no difference anymore in Yr 4 for TLW_i. As expected, the TLW_{plot} was higher in the control plots and lower in the 20% and 40% plots, but the observed difference became smaller from Yr 2 to Yr 4, as the F-value decreased from 22.50 to 6.44. In Yr 4, the 0% plots had caught up with the control, but the 20% and 40% still had a 27% and a 34% lower TLW_{plot}, respectively. As only class 4 and 5 plants were harvested, the treatment itself could have induced changes in size classes for the following years and decreased mean TLW_i in the harvested treatments (see Annex 3 for the proportion of plants in the different size classes as a function of treatments and years). In addition to the results presented in Table 3.2, we performed an additional ANCOVA on the TLW_i data after simulating a harvest of 20% (for the 20% treatment) and 40% (for the 0% treatment) of the total number of bulbs, by removing only classes 4 and 5 plants. This was done to properly compare mean TLW_i in response to differential harvesting, without the statistical artifact caused by the selective harvesting of mid-size plants. This adjustment yielded results similar to those presented in Table 3.2, except that the difference among treatments for TLW_i was no longer significant in Yr 3.

The linear correlations between post-harvest bulb density and the response variables are presented in Table 3.3. There were two significant negative relationships: one with the relative increase of TLW_i (Yr 4/Yr 2) and the other with TLW_i in Yr 4, suggesting that higher densities do affect individual plant growth.

3.5.3 Leaf harvesting

Removing all foliage had a negative effect on BW_d, TLW_d and the probability of producing a floral scape, regardless of the date leaf harvesting took place, compared to the control (Table 3.4). Plants that were subjected to total defoliation still exhibited a lower frequency of bulb division two years later (Yr 3), except for the 20-day group. Surprisingly, the bulb division rate was not significantly affected in Yr 2 ($P = 0.216$). The number of days of growth before leaf harvesting also influenced subsequent growth of wild leek, but the observed differences were significant only when all leaves had been removed. There was thus a significant interaction between the number of leaves harvested and the number of days before harvesting on bulb and leaf size (BW_d and TLW_d) during the subsequent

growing seasons, as illustrated in Figure 3.4. This suggests that delaying the number of days has higher impact when all leaves were harvested than when only half the leaves were removed. Plant survival was not significantly affected by leaf harvesting, regardless of the treatment.

Table 3.3 Correlation matrix between the post-harvest density in Yr 1 and the wild leek growth responses during the subsequent years.

Response variable	Year	Correlation with bulb density in Yr 1(bulb m^{-2})	
		r	P-value
Rel. increase – TLW$_{plot}$[†]	Yr 4/Yr 2	- 0.307	0.145
Rel. increase – TLW$_i$[*]	Yr 4/Yr 2	**- 0.486**	**0.016**
Rel. increase in bulb[‡] number	Yr 4/Yr 2	0.020	0.925
Seeds per bulb	Yr 3	0.145	0.499
Scape per bulb	Yr 3	0.125	0.562
TLW$_i$ (cm)	Yr 2	0.156	0.466
	Yr 3	0.028	0.898
	Yr 4	**-0.537**	**0.007**
TLW$_{plot}$	Yr 2	0.017	0.936
	Yr 3	-0.173	0.420
	Yr 4	-0.181	0.397
Juveniles per plot	Yr 2	-0.034	0.873
	Yr 3	0.012	0.957
	Yr 4	0.055	0.800
Number of bulbs	Yr 2	-0.047	0.826
	Yr 3	-0.130	0.546
	Yr 4	-0.022	0.920

Notes:
Significant linear relations at α = 0.05 are in bold. Bulb density in Yr 1 was assessed immediately after the harvest.
† Relative increase of the sum of TLW$_i$ for the whole plot (TLW$_{plot}$): (TLW$_{plot\_Yr\,4}$ − TLW$_{plot\_Yr\,2}$) / TLW$_{plot\_Yr2}$
* Relative increase of the TLW$_i$: (TLW$_{i\_Yr\,4}$ − TLW$_{i\_Yr\,2}$) / TLW$_{i\_Yr\,2}$
‡ Relative increase of the number of bulbs: (Nb Bulbs$_{Yr\,4}$ − Nb Bulbs$_{Yr\,2}$) / Nb Bulbs$_{Yr\,2}$
Abbreviations: TLW$_i$, mean individual total leaf width ; TLW$_{plot}$, total leaf width of all plants with a TLW$_i$ > 1 cm inside a plot.

Table 3.4 Growth response of wild leek to different leaf harvesting treatments applied once in Yr 1.

Variable	Year	Half of leaves harvested			All leaves harvested			Control	F	P
		15 d.†	20 d.	25 d.	15 d.	20 d.	25 d.			
TLW_d (cm)	Yr 2	10.73 bc (1.58)	11.77 ab (1.58)	11.35 abc (1.57)	7.67 e (1.58)	8.68 de (1.58)	9.99 cd (1.58)	12.91 a (1.54)	30.82	**< 0.001**
	Yr 3	11.65 ab (1.77)	12.04 ab (1.78)	12.53 ab (1.76)	8.31 d (1.77)	9.70 cd (1.77)	10.78 bc (1.77)	13.58 a (1.70)	17.96	**< 0.001**
BW_d (cm)	Yr 1	1.52 bc (0.08)	1.57 b (0.08)	1.63 b (0.08)	1.30 d (0.08)	1.43 c (0.08)	1.56 b (0.08)	1.70 a (0.08)	40.41	**< 0.001**
	Yr 2	1.79 b (0.11)	1.90 ab (0.11)	1.88 ab (0.11)	1.50 d (0.11)	1.63 cd (0.11)	1.74 bc (0.11)	1.97 a (0.11)	21.86	**< 0.001**
	Yr 3	1.56 ab (0.11)	1.55 ab (0.11)	1.62 ab (0.11)	1.28 d (0.11)	1.37 cd (0.11)	1.49 bc (0.11)	1.67 a (0.10)	17.37	**< 0.001**
Scape*	Yr 3	0.189 ab (0.063)	0.188 ab (0.066)	0.197 ab (0.063)	0.034 b (0.022)	0.114 b (0.046)	0.122b (0.048)	0.351 a (0.071)	6.26	**< 0.001**
Division*	Yr 2	0.258 (0.080)	0.303 (0.085)	0.261 (0.077)	0.158 (0.057)	0.217 (0.072)	0.187 (0.064)	0.306 (0.069)	1.39	0.216
	Yr 3	0.198 ab (0.076)	0.270 ab (0.092)	0.303 ab (0.093)	0.104 b (0.050)	0.204 ab (0.077)	0.141 b (0.063)	0.375 a (0.086)	3.57	**0.002**
Survival*	Yr 3	0.830 (0.07)	0.737 (0.09)	0.919 (0.04)	0.831 (0.07)	0.800 (0.08)	0.846 (0.06)	0.863 (0.05)	1.92	*0.076*

Notes:
Numbers in parenthesis are the standard errors.
Within a row, means followed by a same letter are not significantly different at α = 0.05.
† Time treatment: number of days (15d, 20d or 25d) that elapsed between complete leaf unfolding and leaf harvesting
* These values represent the proportion of bulbs that produced a scape, of divided or survived.
Abbreviations: TLW_d, total leaf width of all daughter bulbs issued from a same mother bulb; BW_d,total bulb width of all daughter bulbs issued from a same mother bulb.
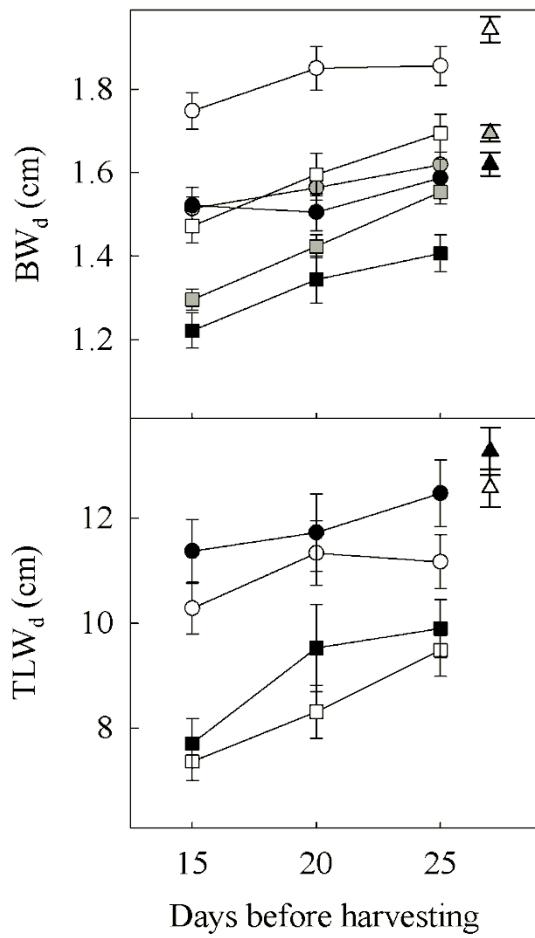
Figure 3.4 Interaction between the effects of the proportion of leaves harvested, the number of days before harvesting and the year elapsed since the harvest took place on TLW_d and BW_d. Δ = 0% leaf harvested (control); $\circ$ = 50% leaf harvested; $\square$ = 100% leaf harvested; grey = Yr 1; white = Yr 2; black = Yr 3. As leaf width in Yr 1 was measured before the treatment, it was not included in the analysis. The controls (0% leaf harvested) are presented for indicative purposes only, and were not included in the ANOVA table calculations of the interactions. Results of ANOVA for BW_d: Nb leaves effect (L) ($F_{1, 1208}$ = 138.4; $P <$ **0.001**), Time (Nb days) effect (T) ($F_{2, 1208}$ = 30.3; $P <$ **0.001**), Year effect (Yr) ($F_{2, 1208}$ = 93.6; $P <$ **0.001**), L × T ($F_{2, 1208}$ = 7.21; $P =$ **0.001**), L × Yr ($F_{2, 1208}$ = 3.27; $P =$ **0.038**), T × Yr ($F_{4, 1208}$ = 0.83; P = 0.504), L × T × Yr ($F_{4, 1208}$ = 0.28; P = 0.891). ANOVA for TLW_d: L ($F_{1, 738}$ = 110.0; $P <$ **0.001**), T ($F_{2, 738}$ = 11.29; $P <$ **0.001**), Yr ($F_{1, 738}$ = 15.7; $P <$ **0.001**), L × T ($F_{2, 738}$ = 5.53; $P =$ **0.004**), L × Yr ($F_{2, 738}$ = 0.02; P = 0.891), T × Yr ($F_{4, 738}$ = 0.17; P = 0.841), L × T × Yr ($F_{4, 738}$ = 0.31; P = 0.734).

3.6 Discussion

3.6.1 Density

Planting wild leek at high density reduced individual growth of both bulb and leaf. This effect is most likely the result of competition and crowding among bulbs. The only other density experiment performed on wild leek, reported no density effect (Ritchey & Schumann, 2005), but the density tested were 11 and 44 bulbs m^{-2}. Their results do not contradict ours: we only observed a density effect on bulb and leaf size at densities of 178 bulbs m^{-2} or more. It nevertheless confirms that planting at density lower than 44 bulbs m^{-2} does not further improve wild leek growth.

The annual yield per m^2 increased with planting density, whereas individual growth and yield per planted bulb decreased with an increase in planting density. This has been reported for commercial onion, for which individual bulb size also decreased with increasing density, but optimal yield per area reached its maximum at an intermediate density (Brewster & Salter, 1980; McGeary, 1985; Herison et al., 1993). Similarly, the leaf area index (LAI) of garlic was previously reported to increase with density, allowing an optimal use of the light available in the field (Moravčević, 2011). A decrease in yield per hectare was observed at very high densities (> 500 bulbs m^{-2}), for bulbs bigger than those of wild leek (McGeary, 1985). The density tested in the present study may not have been high enough to negatively affect yields per area, but the decrease in TLW, BW and annual yield per bulb planted, might reduce the value of the crop on a bulb basis. It is important to mention that the fertilisation was calculated on an area basis (nutrients per area), at rates established for a planting density of 44 bulbs m^{-2} (Bernatchez et al., 2013). Increasing the amount of nutrient applied may improve growth at higher densities. Low density plantation promoted bulb division and flower production. The interaction between density and year on TLW_d attests of the overall faster annual growth of wild leek at lower density. Overcrowding and competition for resources is of concern for this species, considering the very high densities that can be reached in natural populations. Indeed, mortality in the denser patches was reported to occur annually (Nault & Gagnon, 1993). Competition also reduced sexual and asexual reproduction in *Allium oleraceum* L. (Fialová & Duchoslav, 2014).

The density effect was also noticeable in natural populations following partial bulb harvest. The effect only became apparent three years after treatment, as a negative correlation between post-harvest density and TLW_i. In Yr 2 and 3, plants were smaller in the harvested plots than in the control plots, regardless of the percentage of bulb harvested. Plants were thus likely recovering from the transplant stress caused by the harvesting method applied. Similarly, a reduction in individual growth rate has once been reported on wild leek following transplantation (Vasseur & Gagnon, 1994). TLW_i was no longer different between the harvested and the control plots in Yr 4, suggesting a higher growth rate in harvested than in control plots between Yr 2 and 4.

The high mortality recorded on one site reveals the potential deleterious consequences of the harvest method. Harvest on this particular site was performed under a particularly hot and dry day. The stress on the roots may have been enough to affect survival. It is nonetheless surprising, considering the high survival rate reported for transplanted wild leek and its good tolerance to transplantation shock (Vasseur & Gagnon, 1994; Bernatchez et al., 2013). Such transplant stress is usually more common in arid or semi-arid environments (Shinohara & Leskovar, 2014). It would nevertheless be possible to limit post-harvest mortality by (1) harvesting on cool and cloudy days; (2) keeping the plants in a cooler during the process and (3) watering the soil after the bulbs have been replanted. Selective harvest could also take place immediately after leaf senescence at a time when the plants are much less sensitive to transplant shock.

3.6.2 Leaf harvest

As expected, increasing the proportion of leaves harvested and performing an early harvest reduced growth of wild leek during the subsequent years. Allowing the leaves to perform photosynthesis over a longer period of time before being harvested likely permitted more carbon to accumulate in the bulb, whereas removing only half of the leaves likely reduced the loss in carbon and nutrients. It resulted in bigger bulbs at the end of the season the harvest was performed and in the subsequent years, and larger leaves in the following seasons. The significant interaction between the number of leaves harvested and the number of days before harvest suggests that the difference between the harvest of half and all the leaves was less critical if the harvest was delayed as late as possible before

senescence. Indeed, harvesting all leaves after 25 days yields similar growth responses as harvesting half of the leaves.

Removing half of the foliage did not induce complete compensatory photosynthesis on the remaining foliage, since the plants did not accumulate as much C in their bulbs as the control plants, as revealed by the leaf and bulb width measures. Tip-defoliation has been shown to induce compensatory photosynthesis on the remaining leaf section in *Oenothera biennis* L. (Morrison & Reekie, 1995) or to maintain photosynthetic rates constant in damaged leaves of *Agropyron desertorum* (Fish.) J.A. Schutes and *Agropyron spicatum* (now named *Pseudoroegneria spicata* (Pursh) Á. Löve) (Nowak & Caldwell, 1984). Direct measurements of the photosynthetic rates would however be necessary in order to confirm whether or not compensatory photosynthesis occurs following partial leaf harvesting.

Flowering was affected by the proportion of leaf area harvested but not by the timing of defoliation. Flowering might be more sensitive to nutrient than to carbon availability within the bulb. Nault & Gagnon (1988) reported that the presence of chlorophyll in the floral scape allows it to be partially self-sufficient. Moreover, they also observed that reproductive structures contain 30 and 45% of total plant Mg and Ca, respectively, while they account for 15% of the total biomass. Harvesting all leaves also had a negative impact on bulb division. In perennial grassland herbs, defoliation sometimes reduces asexual reproduction by rhizome propagation, but this effect varies with species, as some also had their reproductive effort stimulated by defoliation (Benot et al., 2009; Benot et al., 2010; Bostrom et al., 2013). The proportion of leaf area harvested thus seems to more greatly impact overall growth of wild leek than the time at which harvesting took place.

3.6.3 Stochastic environment

The 2013 growing season appeared to have been harsh on wild leek. It is clearly visible in the leaf harvest experiment as a decrease in BW_d between 2012 (Yr 2) and 2013 (Yr 3), and in the bulb harvest experiment as a decrease in TLW_i from 2013 (Yr 3) to 2014 (Yr 4). Leaf elongation being initiated in autumn (Nault & Gagnon, 1993), TLW is influenced by the previous season. Spring 2013 was particularly dry during wild leek epigeous period: there

was a 23-day period from April 17th to May 9th with only 9.6 mm of rainfall in the Lower Laurentides (Environment Canada, 2013), which even caused early signs of senescence on a few leaves. Similar reduction of leaf longevity during a dry summer has been observed on *Trillium erectum*, another forest herb (Tessier, 2008). This is a good example of how recovery following harvest can be affected by the stochastic environmental conditions that wild leek encounters. Nantel et al. (1996) indeed reduced the recommended bulb harvest rates to compensate for the stochastic environment. Nevertheless, plots harvested at 40% maintain a stable bulb number in the three subsequent years, which suggests that wild leek populations subjected to a high harvest rate can tolerate a harsh season a few years later. A safe advice that can be given to producers is to wait until the population have reached the pre-harvest state, in terms of plant size and number, before harvesting again.

The same precaution can apply to leaf harvesting. For instance, in Yr 2, individuals which had all leaves harvested after 25 days already had on average the same BW_d as the controls in Yr 1 (see Table 3.4), which could suggest that this treatment can be repeated every two years without long term negative effect. However, the growth decline in 2013 after a dry spring suggests waiting somewhat longer. Only long term follow-ups could determine optimal leaf and bulb harvest frequency.

3.6.4 Parasite

This is the first time to our knowledge that *B. guttulatus* is reported on wild leek. This parasite usually affects other root vegetable such as sugar beet, carrot and potato (Allen & Filotas, 2009). *Allium* species produce sulfur volatile compounds that are known to repel, and often directly affect pests, making it more difficult for non-specialists to infect them (Auger et al., 2008; Brewster, 2008). It is possible that another infection weakened the plants, enabling the infection by *B. guttulatus*. Moreover, Nault & Gagnon (1993) reported what they called "Clump death", where most individuals from a wild leek dense patch died within one or two seasons. They suggested that it would be due to a disease, but it was similar to the decline we reported in the density plots. Further research is required to determine if the cause of death is an infection by *B. guttulatus* or if it is preceded by another disease or pest. Lower planting densities are known to slow pest infestations of *Thrips tabaci* on onion (*A. cepa*) (Jima et al., 2013). This could explain the apparent delay

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in the reduction of population size observed at lower density in the present experiment, and constitutes another argument in favor of planting less than 88 bulbs m^{-2}.

3.6.5 Recommendations

The results of the present study allow the following recommendations to be proposed.

1- When establishing a new plantation, the bulbs should be planted at densities which do not exceed 88 bulbs m^{-2}. In the present study, this was the best compromise between individual growth, reproduction and yield per area.

2- Harvesting bulbs in natural populations should be made under cool or overcast days, and the soil watered, in order to prevent mortality. The high survival in most plots indicates that a single high harvest event (up to 40% of bulb harvested) is not a threat to population survival if done under proper conditions. However, we strongly recommend that the following harvest takes place only once the population has reached its pre-harvest density. Applying percentage of harvest such as previously recommended (Nault & Gagnon, 1993; Nantel et al., 1996; Rock et al., 2004) can lead to variable yields considering the very high variability in plant density observed in natural populations. Harvesting down to a fixed post-harvest density between 44 and 88 bulbs m^{-2} should provide optimal regeneration.

3- Leaves should be harvested at the latest possible ($\geq$ 25 days) after complete unfolding. Recreational harvesters should limit to one leaf per plant. Commercial producers, for practical reasons, will harvest all leaves on either patches or rows of plants. This more severe treatment does not affect survival, but the producer should wait until pre-harvest size of the plants is reached before harvesting again, which should take at least two years.

3.7 Acknowledgments

Chapitre 4. Conclusion

4.1 Principaux objectifs de l'étude

Cette étude a permis de répondre à plusieurs objectifs. Le premier était de déterminer l'influence de la composition du couvert forestier et des conditions de lumière induites par celui-ci sur la croissance de l'ail des bois. Une expérience de plantation d'ail sous divers types de couvert forestier, ainsi que des observations en populations naturelles ont été présentées dans le chapitre 2.

Le deuxième objectif était de déterminer l'influence de l'intensité de récolte du feuillage et du moment de cette récolte sur la croissance des plants d'ail des bois. Lors d'une expérience présentée dans le chapitre 3, des feuilles d'ail des bois ont été récoltées à différents moments pendant la saison de croissance, suivant différents taux de récolte.

Le dernier objectif était de quantifier l'impact de la densité des plants d'ail des bois sur sa croissance et sur le rendement total d'une population ou d'une plantation. Les résultats à court et moyen termes d'une expérience de plantation à densité variable sont présentés au chapitre 3. Ce chapitre présente également une expérience de récolte de bulbes qui avait pour but de quantifier la régénération d'une population d'ail des bois suite à une baisse de densité causée par différents taux de récolte.

4.2 Retour sur les hypothèses

L'expérience présentée au chapitre 2 a permis de confirmer partiellement l'hypothèse selon laquelle une plus grande disponibilité de la lumière au printemps devrait favoriser la croissance de l'ail des bois. La quantité de lumière totale transmise au sol durant la saison de croissance a bel et bien un effet positif sur la croissance de l'ail des bois, tant sur la taille du bulbe que sur la production de graines. Toutefois, la lumière traversant la voûte forestière après sa fermeture complète n'a qu'une influence marginale sur la croissance de l'ail. Il s'agit de la mesure de lumière la plus couramment utilisée en recherche sur les plantes forestières, mais elle semble peu applicable à l'étude des éphémères printanières. Afin que le lien entre la lumière totale transmise au sol et la croissance de l'ail soit

significatif, il faut que le calcul de la quantité de lumière tienne compte de la phénologie de la voûte forestière, tel que présenté dans cette expérience.

L'augmentation de la durée de disponibilité maximale de la lumière avant la fermeture de la voûte forestière favorise la croissance de l'ail des bois. La phénologie de la voûte forestière est la composante des conditions de lumière qui influence le plus la croissance de l'ail des bois. Le mécanisme d'action de cet effet serait une relation entre la phénologie de l'ail et celle de l'espèce d'arbre dominant la voûte forestière : l'ail poussant sous une espèce d'arbre à débourrement tardif bénéficie d'une plus longue période de pleine lumière et retarde sa sénescence. Cela permet une plus longue période de fixation de carbone par les feuilles. Un effet similaire a été observé dans le chapitre 3 au cours de l'expérience sur la récolte des feuilles : une période d'activité plus longue avant la récolte des feuilles permettrait à celles-ci de fixer plus de carbone, qui serait transféré au bulbe, permettant ainsi une meilleure croissance de ce dernier. Ainsi, retarder la récolte le plus tard possible en saison est une mesure efficace pour faciliter la régénération des plants. Un tel délai donne l'occasion aux feuilles de fixer une plus grande part de carbone par rapport à ce qui a été fourni par le bulbe pour leur formation, grâce à une plus longue période d'activité photosynthétique.

La même expérience a permis de confirmer qu'un pourcentage de récolte de surface foliaire plus élevé ralentit la croissance les années suivantes. Ne récolter qu'une seule feuille par plant permet en effet de favoriser la régénération de l'ail des bois, en permettant à la feuille restante de continuer la photosynthèse jusqu'à un stade avancé de sénescence. Les nutriments investis dans la feuille sont aussi récupérés lors de la sénescence. Toutefois, récolter les deux feuilles ne met pas en danger la survie du plant, même s'il en réduit davantage la croissance et la reproduction.

Les expériences de densité présentées dans le chapitre 3 ont permis de démontrer que la compétition entre les plants augmente avec la densité des plants, ce qui a un impact sur la croissance individuelle des plants et sur la croissance de la population. Une forte densité a eu l'effet négatif prévu, réduisant la croissance individuelle et l'effort reproducteur des plants. Toutefois, le rendement en biomasse produite par superficie cultivée est plus élevé à forte densité. Cet effet, très clair en plantation, est par contre peu

significatif en populations naturelles. Une grande variabilité dans la microtopographie et les conditions de lumière d'une parcelle à l'autre dans une même population naturelle pourrait reléguer la densité à un facteur d'influence secondaire sur la croissance de l'ail des bois.

4.3 Recommandations et limites de l'étude

4.3.1 Chapitre 2

La principale recommandation pouvant être tirée de ce volet est qu'il est préférable de planter l'ail des bois sous une voûte forestière à débourrement tardif. Cela permet aussi de tirer profit de la diversité d'espèces arborescentes présentes dans une forêt en plantant des espèces qui préfèrent l'ombre tel le ginseng sous les érables et des espèces qui requièrent plus de lumière tel l'ail des bois sous d'autres essences. Les espèces d'arbre du genre *Quercus*, *Fraxinus*, *Tilia* ou *Juglans*, testées dans cette expérience, déploient leurs feuilles très tard au printemps. Toutefois, l'étude ne permet pas de comparer entre elles les différentes espèces d'arbres testées, parce qu'il y avait trop peu de parcelles différentes sous chaque espèce pour effectuer une comparaison statistique. Or, l'influence d'un arbre sur son environnement ne se limite pas à la lumière. Par exemple, la litière formée par un arbre a une grande influence sur les conditions édaphiques (Facelli & Pickett, 1991). La prochaine étape logique serait donc de comparer l'effet des différentes espèces d'arbres à débourrement tardif sur la croissance de l'ail des bois ou d'une autre éphémère printanière d'intérêt, et ce sur une période plus longue afin que le type de litière puisse avoir le temps d'influencer la nutrition minérale de la plante. Néanmoins, le fait que la phénologie de la voûte forestière ait un effet significatif sur la croissance de l'ail des bois malgré la diversité d'essences forestières testées indique qu'il s'agit d'un facteur important.

Cinq parcelles ont été positionnées dans une trouée de la voûte forestière. Cependant, une seule d'entre elles était positionnée de façon à recevoir la lumière directe du soleil à travers la trouée, une fois les feuilles d'arbres déployées. La faible croissance observée dans cette parcelle semble corroborer l'observation générale que la lumière traversant la voûte après sa fermeture n'a qu'un impact marginal sur la croissance individuelle, mais qu'une trop grande ouverture de la voûte peut nuire à la survie et à la reproduction de l'ail des bois. Toutefois, afin de tirer des conclusions fermes par rapport à

l'effet d'une trouée dans la voûte, il faudrait répéter cette expérience en positionnant plusieurs parcelles adéquatement pour recevoir directement la lumière traversant la voûte.

L'analyse de redondance (RDA) effectuée dans ce volet a mis en évidence plusieurs variables d'intérêt ayant un effet potentiel sur l'ail des bois. La variable environnementale la plus importante, autre que la lumière, serait la litière : sa présence en abondance semble favoriser la survie et la division de l'ail des bois, mais pourrait en ralentir la croissance. Cet effet serait lié surtout à la stabilisation de la température du sol. La disponibilité en micronutriments pourrait aussi être un facteur important, tel que montré par le lien entre la balance nutritionnelle [Fe, Mn | Zn, Cu] et la largeur du bulbe ou la production de graines. Finalement, une bonne humidité du sol semble prévenir la sénescence précoce des feuilles d'ail lors de printemps plus secs. Toutefois, le dispositif expérimental ne permet pas de tirer de conclusions fermes quant à ces facteurs environnementaux, parce que les parcelles ont été sélectionnées afin de présenter une grande variabilité de conditions de lumière, tout en réduisant la variabilité environnementale pour les autres facteurs. L'humidité du sol, la disponibilité des micronutriments ainsi que la présence de litière et ses effets sur la température du sol seraient donc trois facteurs intéressants à approfondir dans le cadre de la culture de l'ail des bois. D'ailleurs, les observations menées dans les parcelles naturelles du parc national de la Yamaska indiquent que les variables liées à la lumière expliquent une faible proportion de la croissance de ces populations d'ail. Cela supporte l'hypothèse que d'autres variables environnementales puissent avoir un grand impact sur la croissance de l'ail des bois.

4.3.2 Chapitre 3

Nos résultats montrent une meilleure croissance des plants et un meilleur taux de division et de reproduction pour des densités de plantation de 88 bulbes m^{-2} ou moins. Toutefois, une densité plus élevée permet un meilleur rendement en termes de biomasse produite par superficie cultivée. Dans un contexte de culture commerciale, ce rendement doit être également pris en compte, parce que l'entretien d'une plus grande superficie de culture implique des frais et du temps pour la fertilisation, le désherbage, le travail du sol, etc. De plus, la superficie cultivée devient plus difficile d'accès pour les autres activités d'exploitation de la forêt. Il serait donc important de calculer les coûts et bénéfices pour

différentes densités de culture afin de déterminer la densité qui permet d'optimiser les revenus du producteur.

Les taux de fertilisation appliqués sur les parcelles de cette expérience ont été calculés précédemment sur une base de masse de fertilisants par surface cultivée. Ils ne tiennent pas compte de la variation de densité d'une parcelle à l'autre. De plus, dans le dispositif expérimental ayant servi au calcul de ces taux, la densité de plantation était de 44 bulbes m^{-2} (Bernatchez et al., 2013). Il est possible qu'une augmentation du taux de fertilisation à forte densité permette de compenser en partie la hausse de compétition entre les individus, favorisant ainsi un meilleur rendement.

Les dernières mesures prises en compte dans l'analyse de l'impact de la densité de plantation datent de 2011 et 2012. L'apparition du parasite a trop grandement affecté les parcelles pour permettre de poursuivre l'expérience jusqu'en 2013, tel qu'initialement prévu. Les parcelles les plus denses n'ont connu jusqu'en 2011 qu'une croissance individuelle des plants et peu de division des bulbes. La croissance de ces plants aurait donc fort probablement atteint un plateau dans les années suivantes, si ce n'avait été de l'intervention du parasite. Si cette hypothèse s'avérait exacte, elle s'ajouterait aux arguments en faveur d'une réduction de la densité de plantation.

Le dispositif expérimental de récolte de bulbes n'a pas pu être analysé comme prévu, entre autres parce que la densité en populations naturelles était beaucoup plus variable que prévu. Or, la densité des populations d'ail des bois n'a pas été prise en compte lors des études précédentes qui modélisaient la croissance de la population et proposaient des pourcentages de récolte annuelle durables (Nault & Gagnon, 1993; Nantel et al., 1996; Rock et al., 2004). Si une expérience de récolte en milieu naturel était à refaire, la meilleure approche serait de cibler des densités post-récolte fixes (ex. 50, 100, 200 et 400 bulbes m^{-2}) plutôt qu'un pourcentage (ex. 0, 20 et 40%). Notre expérience a en effet permis d'établir un lien négatif significatif, quoique faible, entre la densité post-récolte et la croissance de l'ail des bois en populations naturelles. En pratique, un exploitant devrait ensuite attendre le retour à la densité pré-récolte avant de récolter à nouveau. Seuls des suivis à long terme permettront d'évaluer le délai d'attente optimal entre les récoltes.

La récolte des feuilles semble un mode d'exploitation durable de l'ail des bois. Dans cette expérience, la survie des bulbes n'a pas été affectée par une récolte appliquée une année seulement, peu importe le moment – de 15 à 25 jours après déploiement des feuilles – et l'intensité de cette récolte. D'un point de vue logistique, cela présente un avantage certain pour un producteur : il est beaucoup plus facile de récolter toutes les feuilles d'une population en même temps, plutôt que de sélectionner une feuille sur deux sur chaque plant. Toutefois, la croissance individuelle et la reproduction des plants s'en trouvent ralenties. Il faudra donc que les récoltes se fassent à intervalles moins fréquents. Pour la récolte récréative, faire l'effort de ne récolter qu'une seule feuille par plant procure néanmoins un net avantage aux plants pendant leur régénération. Dans tous les cas, il est aussi préférable d'attendre le plus tard possible en saison, juste avant la sénescence des feuilles d'ail, avant de les récolter.

La principale limite de cette expérience est qu'elle n'a pas permis d'identifier le mécanisme physiologique qui explique l'effet de la récolte des feuilles sur la croissance subséquente de l'ail des bois. Il serait intéressant d'approfondir cet effet (1) en quantifiant la perte de nutriments occasionnée par la récolte des feuilles; (2) en comparant la quantité de réserves carbonées et azotées accumulées dans le bulbe suite aux différentes intensités et périodes de récolte de feuilles; (3) en déterminant si la coupe d'une seule feuille induit une augmentation du taux de photosynthèse chez la feuille restante. La raison pour laquelle nous n'avons pas appliqué ces mesures est qu'elles sont pour la plupart destructives. Étant donné que, dans ce dispositif, chaque plant représentait une unité expérimentale, leur échantillonnage aurait entraîné la perte de degrés de liberté pour l'analyse statistique. Néanmoins, déterminer si la coupe de feuilles affecte davantage les réserves en carbone ou les réserves en nutriments du bulbe nous permettrait de cibler des interventions pouvant partiellement compenser ces pertes, telle une fertilisation minérale accrue.

Finalement, l'apparition dans nos parcelles du mille-pattes *Blaniulus guttulatus* est inquiétante. À première vue, il semble avoir causé un déclin très rapide des parcelles affectées. Une étude plus approfondie serait nécessaire afin de comprendre l'action de ce parasite et préparer les producteurs à y faire face. Il s'agit à notre connaissance du premier signalement de *B. guttulatus* dans une population d'ail des bois. Son apparition est

surprenante, étant donné qu'il est le plus souvent associé à des cultures telles que la pomme de terre ou la betterave. Les bulbes des *Allium* sont généralement résistants aux parasites. Il serait intéressant d'étudier plus en profondeur les premiers stades d'envahissement par le *B. guttulatus*. Il est possible qu'une autre maladie ait d'abord affecté le bulbe, le rendant plus vulnérable au mille-pattes.

En conclusion, cette étude permet d'émettre les recommandations suivantes concernant la culture de l'ail des bois dans un système agroforestier.

Il est préférable de planter l'ail des bois sous une voûte forestière dense à débourrement tardif, ce qui fournit l'environnement lumineux optimal à sa croissance. Cette plantation devrait être à une densité faible, de 50 à 100 bulbes m^{-2}, afin de limiter la compétition.

La récolte des feuilles devrait se faire le plus tard possible avant leur sénescence. Pour une récolte récréative, ne prélever qu'une feuille par plant réduit les impacts subséquents sur la croissance des plants et aide ainsi la régénération de la plantation ou de la population. Lors de la récolte de bulbes, nous recommandons de replanter suffisamment de bulbes pour atteindre une densité qui favorise la régénération de la population, soit entre 50 et 100 bulbes m^{-2}. Tant dans le cas de la récolte des feuilles que de celle des bulbes, un producteur doit attendre que la population d'ail ait atteint son état pré-récolte avant de récolter à nouveau.

Ces pratiques culturales permettront de favoriser la croissance individuelle des plants, la division des bulbes et la production de graines, afin d'optimiser les rendements à moyen terme d'une culture. Elles sont ainsi un pas de plus dans l'établissement d'une exploitation durable de l'ail des bois.

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Annexes

Annexe 1. Comparaison de différentes méthodes de mesures de la radiation active pour la photosynthèse (PAR) sous couvert forestier.

Méthode	Description	Avantages	Désavantages
Analyse de photos hémisphériques (1, 3, 4, 5)	Prise d'une photo de la voûte forestière à l'aide d'une lentille « fish eye », et calcul de l'ouverture de la voûte forestière à l'aide d'un logiciel (ex. *Gap Light Analyser 2.0, Simon Fraser University, Canada*).	- Permet l'estimation de plusieurs mesures différentes : pourcentage de PAR, variabilité temporelle du PAR, fraction diffuse et directe de la lumière, ouverture de la canopée. - Rapide à utiliser sur le terrain	- Les résultats varient beaucoup d'un analyste à l'autre (subjectivité) - Photos prises sous couvert nuageux, à l'aube ou au crépuscule. - Peu précis dans le cas des couverts forestiers conifériens très denses (3). - Analyse longue
Mesure directe et instantanée sous couvert nuageux (1, 2, 4, 5)	Prise de mesure à l'aide de deux capteurs de lumière, un à découvert et un à l'endroit mesuré. Le rapport entre la mesure sous les arbres et celle à découvert donne une estimation du % de PAR qui atteint le sol à l'endroit mesuré.	- Estimation directe, rapide et facile	- TRÈS dépendant de la météo (mesures prises sous un couvert nuageux total), encore plus que les photos hémisphériques
Capteur de fraction directe (Beam Fraction Sensor) (1)	Appareil qui permet de mesurer la lumière diffuse. En comparant la mesure avec une autre prise à découvert, on peut estimer le % PAR.	- Mesure possible, peu importe la météo - Mesure rapide et facile	- Nécessite l'achat d'équipement coûteux
Méthode standard directe (1, 3, 4, 5)	Capteurs de lumière et enregistreurs laissés sur place toute la saison	- Mesure précise et directe du PAR	- Très coûteux : nécessite 1 à 2 capteurs par parcelle. - Possibilité que le senseur se fasse obstruer ou endommager.
Densiomètre (4)	Outil optique permettant d'estimer directement sur le terrain le pourcentage du couvert forestier caché par les feuilles.	- Faible coût - Résultats fidèles à la réalité - Utilisation sous conditions variables	- Peu précis - Très subjectif - Sujet aux erreurs d'ajustement de son orientation - Faible champ de vision
Analyseur de couvert végétal (LAI-2000, *LI-COR*) (3, 4, 5)	Outil permettant de mesurer l'irradiance selon l'angle de zénith d'où provient la lumière. Il est possible, en prenant plusieurs mesures tout en cachant différentes parties de la lentille, de diviser le ciel en plusieurs sections radiales et de zénith pour une analyse plus précise selon le parcours du soleil.	- Considère la position des trouées du couvert végétal et permet la modélisation selon le parcours du soleil - Analyse rapide - Résultats précis	- Équipement coûteux - Sensible à la lumière directe de la même façon que les photos hémisphériques - Nécessite la prise de plusieurs données sur le terrain afin de diviser le ciel en sections radiales

Sources : (1)Paquette et al. (2007); (2)Parent & Messier (1996); (3)Machado & Reich (1999); (4)Comeau et al. (1998); (5)Gendron et al. (1998)
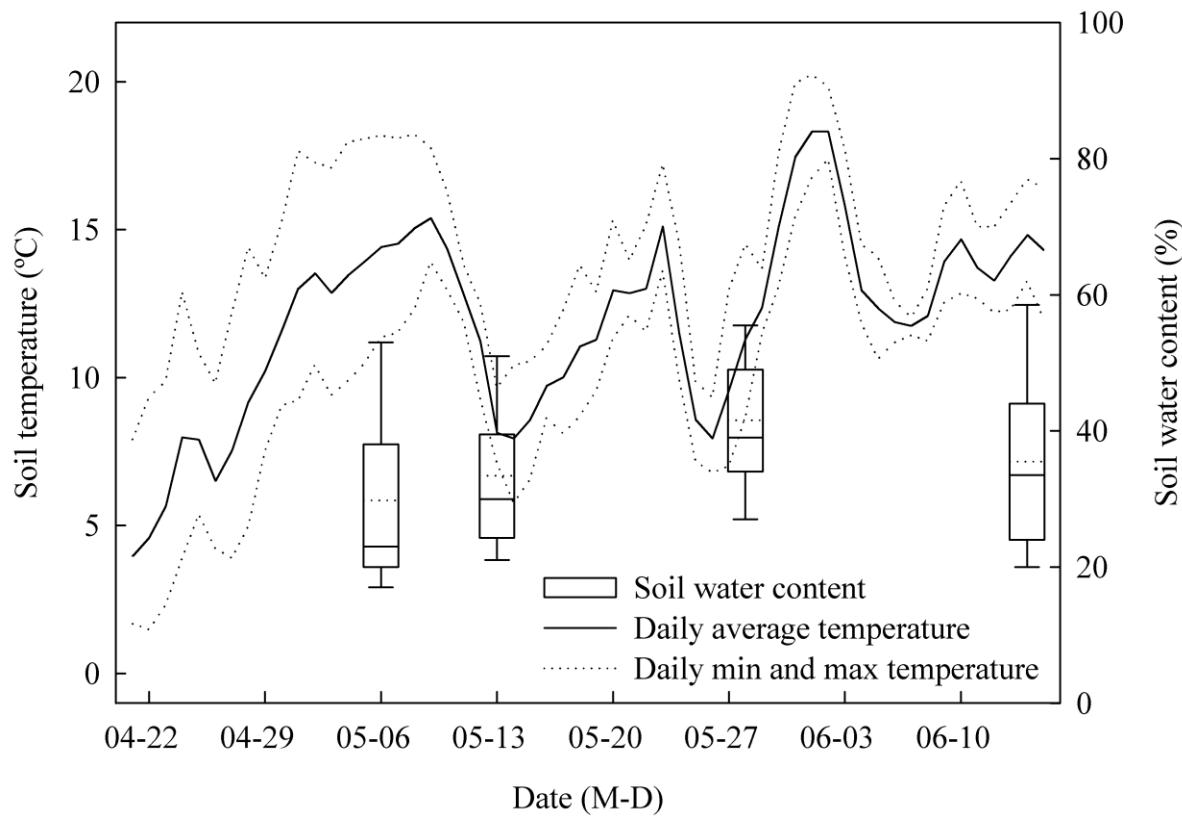
Annexe 2. Average soil temperature at 5 cm depth and soil water content at 10 cm observed in transplant plots throughout the growing season (April 21 to June 15, 2013). Solid line represents the mean temperature for each day averaged among all plots. Dotted lines represent the maximum (day) and minimum (night) temperatures for each day averaged among all plots. Box plots present the range of soil water content observed among all plots on each survey: dotted line represents the mean, the continuous line, the median. See chapter 2 for more details regarding the localisations of the plots.
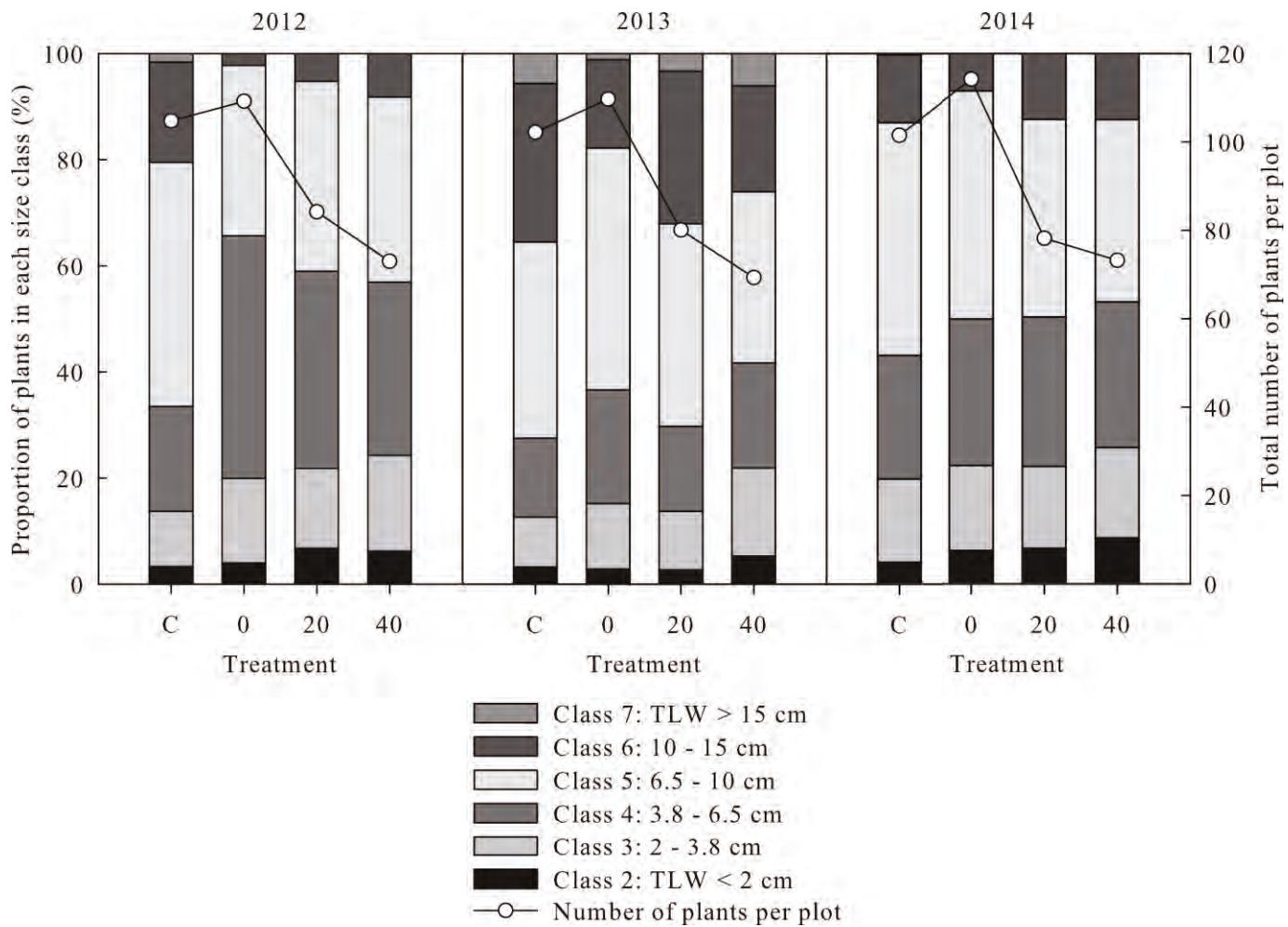
Annexe 3. Proportion of plants in the different size classes varied as a function of harvesting treatments and among years. Treatments were applied in 2011. In 2012 (Yr 2), class 4 appeared under-represented in treatments "20" and "40" compared to "0", probably as a consequence of the harvest, which only included class-4 and -5 plants. In 2013 and 2014 (Yr 3 and Yr 4), class 5 was less abundant in treatments "20" and "40" compared to "0". Size classes are based on total leaf width per plant (TLW) as defined in Nault & Gagnon (1993). The same authors also reported a higher proportion of classes 4 and 5 compared to the smaller and larger class sizes in natural populations. Treatments: C = Control; 0 = all bulbs harvested and replanted; 20 = 20% harvest; 40 = 40% harvest. See chapter 3 for more details.