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VARIACIÓN ALTITUDINAL EN LA ASIGNACIÓN DE CARBONO NO ESTRUCTURAL EN LA ESPECIE HERBÁCEA *Phacelia secunda* EN ANDES DE CHILE CENTRAL

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RESUMEN

No está claro si el aumento observado con frecuencia en los carbohidratos no estructurales (NSC) en plantas expuestas a bajas temperaturas o sequía refleja una mayor sensibilidad del crecimiento que la fotosíntesis en tales condiciones (es decir, limitación del sumidero), o una priorización de la asignación de carbono (C) al almacenamiento. Priorizar el almacenamiento de C a NSC puede favorecer la supervivencia de las plantas en entornos donde la ganancia y la demanda de C a menudo no están sincronizadas. Las plantas alpinas están expuestas a esta asincronía debido a un breve período de ganancia favorable de carbono y un largo período de inactividad invernal seguido de una regeneración al comienzo de la temporada de crecimiento. En gradientes de elevación en regiones con un clima de tipo mediterráneo, como ocurre en los Andes en Chile central, además de la temperatura, la baja disponibilidad hídrica que ocurre a menores elevaciones, también limita la ganancia de C. Así, dada la influencia de la sequía en la fenología del crecimiento y el intercambio de gases en plantas de menor elevación se plantean las siguientes hipótesis: (1) la dinámica estacional de los NSC en los Andes de Chile central cambia con la altitud (2) El almacenamiento de C en plantas de los Andes de Chile central es una prioridad de asignación a expensas del crecimiento, promovida por sequía en bajas altitudes y por frio en altas altitudes y, (3) La asignación de C a almacenamiento en plantas de los Andes de Chile Central de baja elevación es ventajosa para sobrevivir en condiciones de sequía. Para probar las hipótesis utilizó como especie modelo a *P. secunda* creciendo en tres elevaciones diferentes de los Andes de Chile central (1600, 2800 y 3600 m s.n.m.). Se determinó la dinámica estacional de NSC y de crecimiento en plantas de *Phacelia secunda* de dos elevaciones (hipótesis 1). Las concentraciones máximas de NSC se observaron en otoño a los 3600 m y a mediados de verano a 1600 m, indicando que no sólo la temperatura controla la dinámica estacional de las reservas de C en plantas alpinas de biomas con influencia mediterránea. Para dilucidar si el almacenamiento de NSC es prioritario respecto al crecimiento (hipótesis 2), se realizó un experimento de sombreamiento intenso durante un mes y se evaluó la dinámica de recuperación (re-iluminación) del crecimiento y de los NSCs en plantas de las tres altitudes. Se encontró que la re-iluminación provocó un mayor aumento en la concentración de NSC que en la biomasa. Dicho aumento fue de 1,5 y 1,9 veces en las plantas de baja y de alta elevación, respectivamente, sugiriendo diferencias ecotípicas en la asignación de C. Para determinar si los NSCs confieren supervivencia a sequía en plantas de *P. secunda* (hipótesis 3), los individuos de cada elevación fueron expuestos a un experimento factorial con dos temperaturas de crecimiento y tres niveles de riego, y posteriormente evaluados en términos de sobrevivencia y de NSC. Los resultados mostraron que plantas de menor elevación presentaron mayor supervivencia y concentración de NSC frente a sequía, que las plantas de 3600 m, sugiriendo un rol adaptativo de los NSC en plantas de baja elevación. Esta tesis muestra que la asignación de C en *Phacelia secunda* refleja diferencias ecotípicas entre procedencias de elevación determinadas por las condiciones ambientales a las cuales están naturalmente sometidas.

ABSTRACT

It is unclear whether the frequently observed increase in nonstructural carbohydrates (NSCs) in plants exposed to low temperatures or drought reflects a greater sensitivity of growth to photosynthesis under such conditions (i.e., sink limitation), or a prioritization of the repair of carbon (C) to storage. Prioritizing C storage to NSC may favor plant survival in environments where C gain and demand are often out of sync. Alpine plants are exposed to this asynchrony due to a short period of favorable carbon gain and a long period of winter dormancy followed by regeneration at the beginning of the growing season. In elevation gradients in regions with a Mediterranean-type climate, as occurs in the Andes in central Chile, in addition to temperature, the low water availability that occurs at lower elevations also limits the gain of C. Thus, given the influence of drought on growth phenology and gas exchange in lower-elevation plants, the following hypotheses are put forward: (1) seasonal dynamics of NSC in the Andes of central Chile change with altitude (2) C storage in plants from the Andes of central Chile is a priority to protect growth costs, promoted by drought at low altitudes and by cold at high altitudes and, (3) The protection of C to storage in plants of the Andes of Central Chile from low elevation is advantageous for survival in drying conditions. To test the hypotheses obtained as a model species, *P. secunda* grows at three different elevations in the Andes of central Chile (1600, 2800 and 3600 m a.s.l.). The seasonal dynamics of NSC and growth in *Phacelia secunda* plants from two elevations will be developed (hypothesis 1). The maximum concentrations of

NSC were observed in autumn at 3600 m and in mid-summer at 1600 m, indicating that not only temperature controls the seasonal dynamics of C stocks in alpine plants of Mediterranean-influenced biomes. To elucidate whether NSC storage is a priority with respect to growth (hypothesis 2), an intense shading experiment was carried out for one month and the dynamics of recovery (re-illumination) of growth and NSCs in plants of the three plants was evaluated. altitudes. Re-illumination was found to use a greater increase in NSC concentration than in biomass. This increase was 1.5 and 1.9 times in the low and high elevation plants, respectively, suggesting ecotypic differences in the exposure of C. 3), the individuals of each elevation were exposed to a factorial with two temperatures of growth and three levels of irrigation, and subsequently evaluated in terms of survival and NSC. The results showed that plants from lower elevations presented higher survival and concentration of NSC against drought, than plants from 3600 m, suggesting an adaptive role of NSC in low elevation plants. This thesis shows that the protection of C in *Phacelia secunda* reflects ecotypic differences between elevational provenances determined by the environmental conditions to which they are naturally subjected.

INTRODUCCIÓN GENERAL

En plantas superiores, una fracción importante del carbono (C) fijado durante la fotosíntesis es inmediatamente metabolizado para proporcionar energía en las distintas funciones vitales tales como el crecimiento, mantenimiento, reproducción, y defensa. La fracción de C que no es metabolizado de manera inmediata puede acumularse en las hojas para cumplir roles osmóticos o bien exportarse desde el mesófilo, principalmente en forma de sacarosa, a otros órganos de la planta para ser almacenados (Martínez-Vilalta et al. 2016; Taiz and Zeiger 2003). Este transporte ocurre en el floema, parte del sistema vascular donde se mueven los carbohidratos desde el tejido fotosintético (fuentes) hacia áreas de almacenamiento (sumideros) y, posteriormente, desde éstas áreas hacia tejidos donde los carbohidratos son demandados. El carbono se almacena principalmente como almidón y azúcares de bajo peso molecular (NSC, del inglés non-structural carbohydrates; Li et al. 2008; Li et al. 2002), dependiendo de la especie y órgano de la planta (Chapin et al. 1990). El almidón es el mayor compuesto de almacenamiento en plantas superiores, mientras que los azúcares de bajo peso molecular cumplen funciones de almacenamiento, transporte, osmoprotección, y osmorregulación, entre otras. Además, muchas plantas almacenan fructanos (Livingston III et al. 2009; Van den Ende et al. 2004) (Hendry 1993). Para los fructanos, tres funciones principales se han abordado en la literatura, reserva de C (Archbold 1940; Smith 1972), compuesto que aumenta la tolerancia de las plantas a la sequía y al congelamiento (De Roover et al. 2000; Kawakami et al. 2008; Livingston III et al. 2009; Pilon-Smits et al. 1995; Salinas et al. 2016) y estabilizador de membranas celulares (Hincha et al. 2007). La acumulación de fructanos en las plantas ha sido relacionada con una mayor supervivencia bajo estrés como frío y sequía (Livingston III et al. 2009). El almacenamiento de C puede representar hasta un 50% del peso seco de las plantas (Chapin et al. 1990), o incluso más en las especies que pasan ciertos períodos de su ciclo de vida en forma de órganos de reserva. Las reservas de C son raramente agotadas en condiciones naturales y a nivel global (Martínez-Vilalta et al. 2016), sugiriendo que son fundamentales para la vida de las plantas. Asimismo, las reservas de C confieren supervivencia frente a estrés biótico y abiótico, promoviendo el reemplazo de tejido en respuesta a la defoliación y al fuego, y permitiendo funciones vitales durante períodos prolongados de estrés hídrico (Signori-Müller et al. 2021) . Estudios han indicado un importante rol de los NSC en la supervivencia. Plantas con alto contenidos de NSC tienen mayor probabilidad de supervivencia, tal como lo demuestran Barker Plotkin et al. (2021), quienes en un gradiente natural de severidad de defoliación encontraron que existe un umbral de NSC para la supervivencia de los robles maduros. Bajo ese umbral, la mortalidad es muy probable.

Los NSC son almacenados en tallos, ramas, bulbos, semillas y raíces, entre otros. En plantas perennes de climas templados, los NSC son almacenados hacia finales de la estación de crecimiento, y son utilizados durante el período de latencia invernal, donde la ganancia de carbono es escasa o nula y más significativamente, durante el período posterior a este, cuando se reactiva el crecimiento (Fig. 1). En climas con estación seca, los NSC son utilizados durante el período de mayor restricción hídrica, cuando la fotosíntesis está limitada por cierre estomático o por senescencia foliar. Por ejemplo, en las zonas tropicales con estación seca, muchas plantas pierden sus hojas, dependiendo durante este período totalmente de sus reservas de C (Palacio et al. 2012). En algunas especies leñosas de hoja caduca de clima templado la floración se produce antes del desarrollo vegetativo, siendo altamente dependiente de las reservas de C (Fowells 1965). En conclusión, los patrones de acumulación y uso estacional de los NSC en plantas de climas templado, sugieren un rol primordial del almacenamiento del C para la vida de las plantas en estos ambientes.

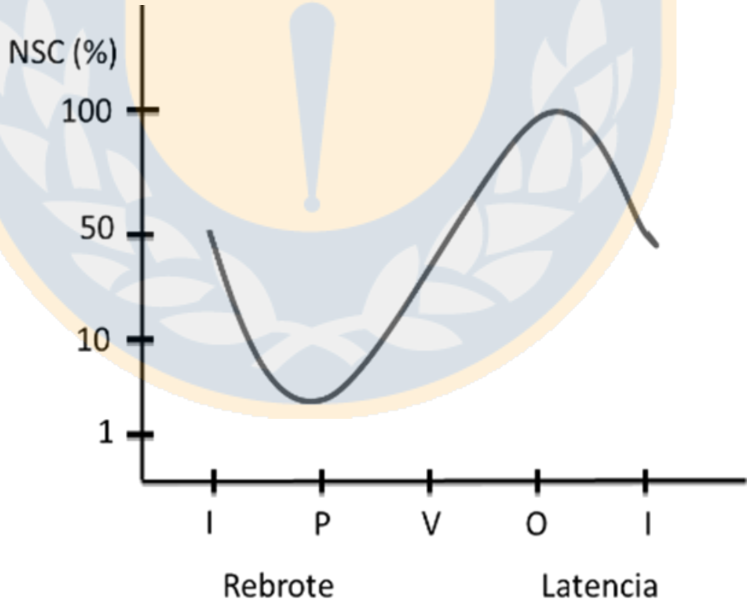

Figura 1 Variación temporal de carbohidratos no estructurales (NSC) en plantas perennes alpinas de climas templados basada en Midorikawa (1959), Mooney and Billings (1960), Hadley and Bliss (1964), Shibata and Nishida (1993), y (Wyka 1999)

Dinámica de los NSC en respuesta a bajas temperaturas y sequía

Las concentraciones de NSC son indicadores de los procesos que limitan el crecimiento de las plantas (Hoch 2015). Un aumento en las concentraciones de NSC junto con una disminución en las tasas de crecimiento durante el período de crecimiento, sería indicativo de que no existe limitación por C sino de impedimentos directos sobre el proceso de crecimiento (ej. división y elongación celular; “limitación de sumidero”). Por el contrario, un crecimiento limitado junto con una disminución en la concentración de NSC, sería indicativo de que el crecimiento de la planta está siendo limitado por C (limitación de fuente). En plantas que crecen en condiciones de bajas temperaturas o sequía, se ha encontrado acumulación de NSC junto con disminución de crecimiento durante la estación de crecimiento (Fatichi et al. 2014; bajas temperaturas: Hoch and Körner 2003; Hoch and Körner 2009; Körner 2015; sequía: Zhang et al. 2015), sugiriendo limitación por sumidero. Dicha acumulación se debería a que tanto la sequía como las bajas temperaturas ejercen mayores restricciones sobre el crecimiento que sobre la fotosíntesis (Boyer 1970; Domisch et al. 2001; Kontunen-Soppela et al. 2002; Körner 2015; Muller et al. 2011); (Bajas temperaturas: Fig. 2; Sequía: Fig. 3). Por otro lado, existen estudios que indican que la sequía y las bajas temperaturas sí podrían causar limitación por C. Por ejemplo, en un estudio realizado en los Alpes Suizos, Dawes et al. (2013) encontraron que *Larix decidua* Mill. aumentó su crecimiento en respuesta a un aumento en la concentración de CO_2 ambiental aplicado durante nueve años. Dawes et al.

(2011), realizaron el mismo experimento con arbustos enanos alpinos, encontrando que *Vaccinium myrtillus* L. mostró un claro efecto positivo del aumento de CO_2 sobre el crecimiento. Similarmente, Guehl et al. (1994), encontraron que plántulas de *Quercus petraea* (Mattuschka) Liebl. expuestas a sequía aumentaron su biomasa en un 46% en respuesta a un aumento en la concentración de CO_2. También consistente con la hipótesis de limitación por C, Fajardo and Piper (2014) encontraron que plántulas de *Nothofagus pumilio* (Poepp et Endl.) Krasser disminuyeron su crecimiento y sus concentraciones de NSC cuando fueron expuestas experimentalmente a bajas temperaturas. En conclusión, aún no existe claridad sobre el mecanismo que limita el crecimiento en plantas expuestas a bajas temperaturas y sequía; en particular, si el menor crecimiento se debe a limitación por sumidero o por fuente.

Una explicación alternativa a la limitación por C para el aumento de la concentración de NSC durante la estación de crecimiento en condiciones de sequía y bajas temperaturas, es que la acumulación de NSC se deba a un cambio en la asignación de C desde crecimiento hacia almacenamiento. Esta hipótesis propone una priorización del almacenamiento a costa de crecimiento, implicando que el C asimilado sea un recurso limitante para el crecimiento de las plantas, aun cuando las reservas de C sean altas (Sala et al. 2012; Smith et al. 2003; Wiley and Helliker 2012). Esta hipótesis además significa la existencia de competencia por C entre los dos sumideros (crecimiento y almacenamiento) (Wiley and Helliker 2012), y por lo tanto, se opone a la interpretación de que el aumento en las concentraciones de NSC en condiciones de sequía o frío refleja acumulación, es decir una mera consecuencia de limitaciones directas sobre el crecimiento que impiden la inversión de C. La priorización en la asignación de C a almacenamiento podría ser ventajosa en plantas de ambientes fríos o con limitaciones hídricas, donde habitualmente existe un asincronismo entre la ganancia y la demanda de C. Por ejemplo, en ambientes con clima mediterráneo, las plantas deben sobrevivir largos períodos de baja ganancia de carbono debido a sequía. La utilización de reservas de C acumuladas en períodos favorables resultaría muy ventajosa para sobrevivir los períodos de sequía. Similarmente, en ambientes alpinos, donde la estación de crecimiento es muy corta (Körner 2003a), las plantas suelen estar expuestas a perturbaciones naturales que limitan su ganancia de C (granizo, heladas tardías, deslizamiento de terreno, nevadas y heladas estivales, períodos con escasa radiación lumínica, etc) (Körner 2003a). En estos ambientes, depender sólo de la fotosíntesis para realizar diferentes funciones (crecimiento, respiración, reproducción, defensa, etc.), podría ser muy riesgoso.

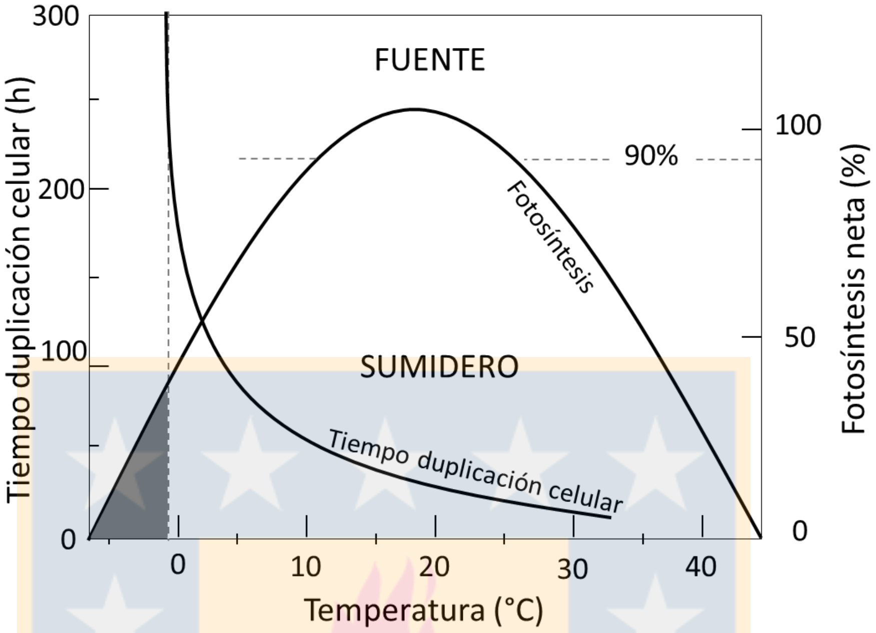

Figura 2 Sensibilidades relativas de la fotosíntesis neta (actividad fuente) y la actividad meristemática (actividad sumidero, expresada como tiempo de duplicación celular) a la temperatura. Modificado de Körner (2013).

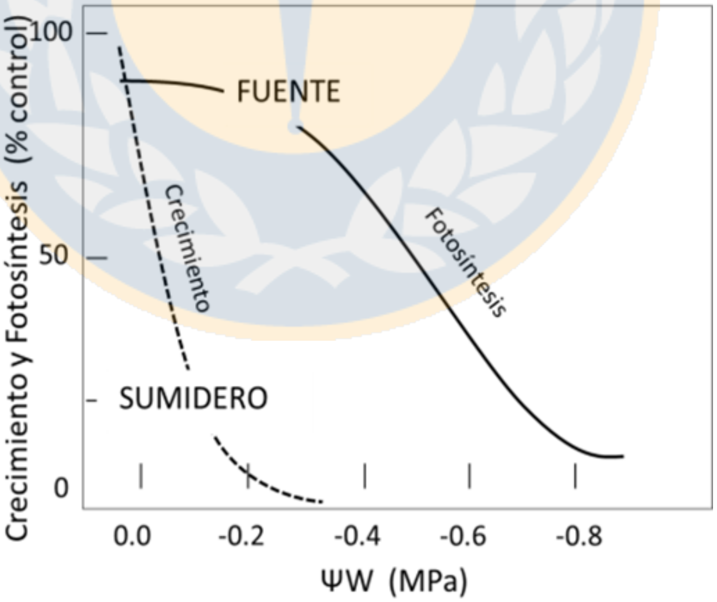

Figura 3 Impactos del estrés hídrico sobre la fotosíntesis y el crecimiento de las plantas. Adaptado de Muller (2011) y Körner (2013).

Asignación de C en plantas alpinas

Los ambientes alpinos se caracterizan por presentar bajas temperaturas de aire y suelo, fuertes vientos, altos niveles de radiación, baja cantidad de nutrientes y una muy corta estación favorable para el crecimiento (Körner 2003a). A medida que aumenta la elevación ocurren particularmente dos cambios ambientales importantes: (1) disminuye la temperatura ambiental (Körner 2003a) y (2) disminuye la estación favorable para el crecimiento (Körner 2007). Esto implica que las especies vegetales que allí habitan deban completar sus eventos fenológicos de manera muy rápida (Bliss 1956). En plantas perennes de ambientes de montaña, se ha encontrado una variación estacional muy marcada de NSC (Mooney and Billings 1960; Wyka 1999), junto con altas concentraciones de los mismos (Chapin et al. 1990; Mooney and Billings 1960; Scott and Billings 1964). Los mecanismos causales de estas dos características no están claros.

La marcada variación estacional de los NSC se asocia a una marcada estacionalidad climática. En los hábitats de altas elevaciones y latitudes templadas, la estacionalidad es particularmente notoria. Durante el invierno, las plantas alpinas permanecen inactivas bajo la nieve, sobreviviendo a expensas de sus reservas de C (Fonda and Bliss 1966). A este período le siguen condiciones mucho más favorables para el crecimiento y la fotosíntesis debido a las temperaturas más cálidas y al derretimiento de la nieve en primavera (Körner 2003a). Durante este período, las demandas de C para el crecimiento también se satisfacen en gran medida mediante la movilización de las reservas de C (Fonda and Bliss 1966). Esta variación está dada por disminución de NSC durante la latencia (invierno), llegando a niveles mínimos en la primavera, seguido de la reposición gradual tan pronto como se restablece una cantidad suficiente de follaje fotosintético (ver referencias en introducción del capítulo 1). La dinámica de la concentración de NSC en plantas alpinas está caracterizada por un balance negativo de C al comienzo de la temporada de crecimiento, cuando las plantas deben reconstruir nuevos tejidos después del letargo invernal. Esto es particularmente notable en las plantas herbáceas pequeñas que pierden la mayor parte de su biomasa aérea durante el invierno. De hecho, la dinámica estacional de NSC en plantas herbáceas es la más pronunciada en comparación con otros tipos funcionales de plantas (Martínez - Vilalta et al. 2016).

Las altas concentraciones de NSC observadas en plantas alpinas podrían relacionarse a la imprevisibilidad climática (Monson et al. 2006; Mooney y Billings 1960; Wyka 1999). Las restricciones ambientales, como la corta temporada de crecimiento, eventos impredecibles de heladas y otros peligros naturales como nevadas, deslizamientos de tierra y períodos prolongados de baja radiación solar que caracterizan los ambientes alpinos (Körner 2003a), podrían conducir a limitaciones severas de C y potencialmente a la inanición de C. Además, las plantas alpinas desarrollan nuevo follaje tan pronto como la nieve se derrite, dependiendo de los recursos almacenados durante la temporada de crecimiento anterior, como se ha descrito en el apartado anterior. Tanto los eventos de congelación al principio como al final de la temporada causan un cese abrupto de la ganancia de C debido al daño foliar y, por lo tanto, reducen la duración efectiva de la temporada de crecimiento (Körner 2003a). Dado que estas perturbaciones a menudo son impredecibles en la mayoría de los ecosistemas alpinos, depender solo de la fotosíntesis actual para satisfacer las demandas de C de diferentes sumideros (crecimiento, respiración, reproducción, defensa, etc.) podría ser arriesgado. Por lo tanto, las plantas alpinas podrían tener una estrategia conservadora de C, manteniendo altos niveles de almacenamiento de C durante la temporada de crecimiento y priorizando la asignación de C para el almacenamiento después de un período de limitación severa de C o inanición de C. De acuerdo con esta hipótesis, se ha observado que las plantas alpinas mantienen una alta concentración de NSC aún en los períodos de máximo crecimiento (García-Lino et al.2017; Monson et al.2006; Wyka 1999). El almidón, los azúcares de bajo peso molecular y los fructanos son las principales fracciones de NSC observadas en plantas alpinas en condiciones de baja temperatura (Chatterton et al.1989; García-Lino et al.2017; Guevara-Figueroa et al.2015; Valluru y Van den Ende 2008).

Las restricciones ambientales, como la corta temporada de crecimiento, eventos impredecibles (e.g. heladas, deslizamientos de tierra), períodos prolongados de baja radiación solar o un largo período bajo nieve, sugieren que la priorización de la asignación de C a almacenamiento en desmedro del crecimiento podría ser ventajosa en plantas alpinas. En línea con esta idea, las plantas alpinas presentan generalmente baja estatura a pesar de sus altas capacidades fotosintéticas. Esta priorización podría favorecer la supervivencia durante la latencia invernal, la reactivación del crecimiento primaveral y la resistencia a disturbios impredecibles durante la estación de crecimiento. Considerando que la temperatura y la estación de crecimiento disminuyen con la elevación, se podría esperar que la priorización en asignación de C a almacenamiento sea mayor en plantas de mayor elevación que en aquellas de sitios más bajos.

Asignación de C en plantas alpinas de clima Mediterráneo

Los climas mediterráneos se caracterizan por veranos cálidos y secos, e inviernos fríos y lluviosos. En montañas de regiones con clima mediterráneo, la precipitación generalmente aumenta con la elevación (Piper et al. 2016). La menor precipitación y mayor temperatura que caracteriza a las menores elevaciones expone a las plantas a una mayor duración y severidad de sequía estival (Piper et al. 2016). En ambientes mediterráneos, la sequía estival es una limitación principal para el rendimiento y la supervivencia de las plantas. Como resultado de la sequía estival, las altas temperaturas y la alta radiación, las plantas alpinas de regiones con clima mediterráneo experimentan disminuciones en la fotosíntesis y aumento del fotodaño a medida que transcurre la estación de crecimiento (Hernández-Fuentes et al. 2015). Estas limitaciones fotosintéticas son especialmente marcadas en el extremo inferior del gradiente de elevación, tal como se observa en las hierbas perennes de los Andes chilenos (Sanfuentes et al. 2012, Hernández-Fuentes et al. 2015). Por ende, el poco C asimilado durante la sequía, podría ser insuficiente para cubrir las demandas de las diferentes funciones metabólicas y osmóticas a lo largo de la estación seca, mientras que contar con altas concentraciones de

NSC antes del período seco podría ser beneficioso, resultando en un aumento de la supervivencia en éstas plantas. Consistente con esta premisa, se ha encontrado que árboles de montañas de regiones con clima mediterráneo ubicados en elevaciones inferiores presentan mayor re-movilización de NSC durante el verano que árboles de elevaciones superiores (Piper et al. 2016). En la misma línea, las plantas alpinas de clima mediterráneo expresan rasgos fisiológicos que les permiten prosperar en tales condiciones. Por ejemplo, acumulan compuestos que incrementan el potencial osmótico tales como prolina que mejoran el rendimiento fotosintético en condiciones de sequía (Hayat et al. 2012) y presentan mayor eficiencia en el uso del agua (Magaña Ugarte et al. 2019). Considerando que en la mayoría de las montañas de ambientes mediterráneos la limitación hídrica es más severa en menores elevaciones, se podría esperar que exista una alta concentración de NSC en plantas de menor elevación, lo cual se debería a una priorización de almacenamiento por sobre crecimiento.

Problema de estudio y *Phacelia secunda* como modelo

En los Andes de Chile central la sequía es un factor limitante en la ganancia de C de las plantas, especialmente en elevaciones menores (Hernández-Fuentes et al. 2015; Sanfuentes et al. 2012). Esto se debe a que los Andes de Chile central se encuentran bajo clima mediterráneo, con escasas precipitaciones durante la temporada de crecimiento las cuales generan condiciones de severa escasez de agua (c.a. 5 meses de sequía, Cavieres et al. 1998). Por lo tanto, mientras en regiones montañosas de clima no mediterráneo la duración de la estación de crecimiento disminuye con la elevación (Körner 2003a), la sequía asociada a clima mediterráneo podría alterar este patrón acortando la estación de crecimiento a menor elevación. Así, en los Andes de Chile central la estación de crecimiento a bajas elevaciones podría ser tan breve como a mayores elevaciones. Además, podría existir una diferencia en el período del año en que ocurre la estación de crecimiento, siendo más anticipada a menores elevaciones. Dada la similitud en duración de estación de crecimiento entre altas y bajas elevaciones en los Andes de Chile central, y la presencia de condiciones ambientales restrictivas para la ganancia de C en ambas elevaciones (bajas temperaturas en alta elevación; sequía en baja elevaciones), una priorización de asignación de C a almacenamiento en desmedro del crecimiento podría ser ventajoso para la sobrevivencia de las plantas en ambas elevaciones.

En los Andes de Chile central, varias especies presentan una amplia distribución altitudinal (Cavieres et al. 2000), estando así expuestas a diferentes condiciones restrictivas. Entre estas especies se encuentra *Phacelia secunda* J.F. Gmel. (Boraginaceae). A diferencia de las otras especies con amplia distribución en Chile, *P. secunda* se desarrolla exitosamente en climas muy diferentes como son el clima costero, el clima tipo mediterráneo del valle Central y el clima alto-andino (Cavieres 2000). *Phacelia secunda* es una hierba perenne de baja estatura que posee una raíz profunda que puede alcanzar a medir más de 50 cm en los Andes de Chile central *P. secunda* se distribuye entre los 1.600 y los 3.600 m s.n.m., presentando variaciones morfológicas a lo largo del gradiente altitudinal en forma “clinal”

(Briggs and Walter 1997) en respuesta a las modificaciones del ambiente (Cavieres 2000). Por otro lado *P. secunda* es una especie hemicriptófita facultativa con respecto al tiempo de muerte foliar. A menores elevaciones *P. secunda* entra en dormancia inmediatamente después de fructificar, mientras que a mayores elevaciones continúa su crecimiento vegetativo hasta inicio de otoño, luego del período reproductivo. De acuerdo a los antecedentes descritos, *P. secunda* es un buen modelo de estudio para abordar las siguientes preguntas: ¿es la dinámica estacional de NSC en *P. secunda* a lo largo del gradiente altitudinal de Chile central afectada sólo por la temperatura, o también por la presencia de sequía estival? ¿presenta diferentes dinámicas estaciones de NSC en elevaciones contrastantes?, ¿son las concentraciones de NSC en *P. secunda* resultado de limitación por sumidero o de una priorización de asignación de C a almacenamiento versus crecimiento? ¿Representan los NSC una ventaja para sobrevivir ante condiciones de bajas temperaturas y sequía?

Para abordar estas preguntas se plantearon las siguientes hipótesis

Hipótesis

1.- Dadas las condiciones contrastantes entre elevaciones, la dinámica estacional de los NSC en *P. secunda* cambia con la elevación: A mayor elevación, el máximo estacional de NSC ocurre en otoño, mientras que, a menor elevación, ocurre a mediados del verano. Lo anterior genera a su vez, que la temporada de crecimiento en plantas de menor elevación, sea tan breve como en elevaciones más altas.

2.- El almacenamiento de C es una prioridad con respecto al crecimiento en plantas de elevaciones bajas y altas donde la sequía y el estrés por frío son mayores, respectivamente.

3.- La asignación de C a almacenamiento en *P. secunda* de los Andes de Chile central de baja elevación es ventajosa para sobrevivir en condiciones de sequía.

Objetivo general y objetivos específicos

Objetivo general:

Estudiar las consecuencias del clima mediterráneo en el almacenamiento de C en la especie *P. secunda* en el gradiente altitudinal de los Andes de Chile central.

Objetivos específicos:

Objetivo 1: Caracterizar la dinámica estacional de NSC en plantas de *P. secunda* establecidas en diferentes elevaciones.

Objetivo 2: Dilucidar si el almacenamiento de C se debe a una prioridad de asignación a reservas o sólo es una consecuencia de un crecimiento limitado.

Objetivo 3: Determinar si el almacenamiento de C confiere sobrevivencia a sequía en *P. secunda,* especialmente en individuos de menor elevación.

CAPÍTULO I ELEVATIONAL VARIATION OF THE SEASONAL DYNAMIC OF CARBOHYDRATE RESERVES IN AN ALPINE PLANT OF MEDITERRANEAN MOUNTAINS

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Abstract

In alpine plants, the temporal variation in the concentrations of non-structural carbohydrates (NSC) is closely related to the growth phenology, which is largely controlled by annual variations in temperature. However, in alpine areas of Mediterranean-type climate regions, plants growing at low elevations are also exposed to seasonal drought. Given the influence of drought on growth phenology and gas exchange, we hypothesize that the seasonal dynamics of growth and NSC concentrations in alpine plants of Mediterranean biomes is co-controlled by elevational gradients of temperature and soil moisture. If so, the end of the growing season and the maximum NSC concentrations at lower elevations should coincide with the occurrence of drought. We characterized the seasonal dynamics of photosynthesis capacity, growth and NSC concentrations, in an alpine plant species of the Andes of central Chile (*Phacelia secunda* J.F. Gmel.) at 1600 and 3600 m. We found that the length of the growing season was similar between elevations, but the timings differed. Whilst at 3600 m, the number of leaves and the mean leaf length progressively increased from December to February, at 1600 m, in contrast, they increased from the October to December. Likewise, maximum NSC concentrations at 3600 were observed at autumn along with growth cessation. Conversely, at 1600 m, the highest NSC concentration and the growth cessation were found towards mid-summer, and coincided with a drastic drop in both stomatal conductance and photosynthesis which were not observed at 3600 m. These results demonstrate that temperature alone does not control the growth phenology and the seasonal dynamics of NSC concentrations in alpine plants of Mediterranean biomes. Rather, summer drought also exerts a significant influence in the timing of the growing season and the NSC dynamics.

Key words*: Andes, central Chile, carbon storage, non-structural carbohydrates, Phacelia secunda.*

Introduction

Plants fix carbon (C) through photosynthesis and use it to provide the energy for growth, maintenance, reproduction, and defense, amongst other processes (Chapin et al. 1990). Nonetheless, a significant fraction of fixed C is allocated to storage in the form of non-structural carbohydrates (NSC), which include low molecular weight sugars, starch, and fructans (Chapin et al. 1990). For most plant species, starch represents the main form of C reserves. However, fructans and low molecular weight sugars are quantitatively and functionally important in herbaceous plants (Reyes-Bahamonde et al. 2021; Suzuki 1989). For example, fructans have been described as one of the main forms of stored energy in ca. 15% of the angiosperms, being particularly relevant in Asteraceae, Campanulaceae, and Boraginaceae amongst dicots, and in Poaceae and Liliaceae amongst monocots (Livingston III et al. 2009; Van den Ende et al. 2004). Non-structural compounds other than carbohydrates, like lipids and sugar alcohols may be important C storage forms in other plant species like conifers (Hendry 1993a; Murphy 1993). Non-structural C compounds can be used when the C balance between C input and C demands is negative (Hoch 2015; Sala et al. 2010). A negative C balance can occur under different stressful conditions such as shade, drought and after disturbances such as fire or herbivory (Sala et al. 2010). In seasonal climates, like temperate and Mediterranean climate, a negative C balance occurs at the beginning of the growing season, when plants must rebuild new tissues after the winter dormancy (Hoch 2015; Piper 2020). This is particularly remarkable in small herbaceous plants that lose most of their aerial biomass during winter. Indeed, the NSC seasonal dynamics in herbaceous plants is the most pronounced when compared to other plant functional types (Martínez-Vilalta et al. 2016). Despite its quantitative importance, the dynamics of NSC in alpine plants remain little understood.

In high elevations habitats (hereafter alpine habitats) of temperate latitudes, seasonality is particularly conspicuous. During winter, alpine plants remain dormant under snow, surviving at the expenses of their C reserves (Fonda and Bliss 1966). This period is followed by much more favorable conditions for growth and photosynthesis due to the warmer temperatures and snow melt in spring (Körner 2003). During this period, the C demands for growth are also largely met by C reserves remobilization (Fonda and Bliss 1966). For example, in alpine environments of the Rocky Mountains, the NSC content of *Oxytropis sericea* declined by 50% during winter dormancy, suggesting that the dormant period constituted the greatest sink for stored carbohydrates (Wyka 1999). In the same alpine region, Mooney and Billings (1960) found that in three alpine plant species a significant part of the stored carbohydrate in underground organs was used in growth prior to snowmelt and during the period of shoot growth immediately following snowmelt. In the alpine herb *Polygonum bistortoides*, 50% of the rhizome reserves were used in a single week during early growth (Mooney and Billings 1960). These studies show a high consistency in the temporal variation of NSC concentrations in herbaceous plants from temperate alpine habitats, even so plants do not deplete all their reserves during the period of greatest seasonal asynchrony between C gain and C demands. The seasonal dynamics of NSC storage in alpine plants is closely related to growth phenology, which is largely controlled by temperature (Fonda and Bliss 1966). However, for alpine plants of Mediterranean biomes we could expect a strong influence of drought on both growth phenology and NSC dynamics. This possibility has been little examined.

Mountains located in Mediterranean type regions are characterized by warm and dry summers (Cavieres et al. 2006; Piper et al. 2016). In these mountains, plants growing at low elevations are exposed to drought (Magaña Ugarte et al. 2019; Piper et al. 2016), which may cause growth cessation, stomatal closure and reduction or inhibition of photosynthesis (Hernández-Fuentes et al. 2015). For instance, Hernández-Fuentes et al. (2015) found that in the alpine plant *Phacelia secunda* growing in Central Chile photosynthesis and stomatal conductance were more than 6-fold lower at 1600 m a.s.l than at 3600 m a.s.l., respectively. Given the influence of drought on growth phenology and gas exchange, it can be expected that drought affects the seasonal dynamics of NSC in alpine plants along an elevational gradient. Further, because of the summer drought, the growing season at low elevation could be as brief as that at higher elevations, and the C reserves could be important not only to support metabolism during winter and early spring, but also during the severe summer drought and afterwards. Mooney and Billings (1965) showed that in the Sierra Nevada of California, a Mediterranean mountain with a conspicuous summer drought, low elevation alpine plants contain similar NSC levels as conspecific plants from higher elevations. Indeed, *Polygonum bistortoides* showed higher NSC levels at lower than at higher elevations during spring, suggesting that low elevation plants accumulate significant NSC levels before the beginning of the dry period which are in turn used during the long period of summer drought, the subsequent winter dormancy, and the early spring (Mooney and Billings 1960). As a result, maximum growth and NSC concentrations should occur earlier at lower than at higher elevations.

The opposite gradients of drought and temperature affecting the elevational distribution of alpine plants in Mediterranean mountains could also determine differences in the seasonal dynamics of the NSC fractions between high and low elevation plants. The accumulation of both low molecular weight sugars and fructans has been described as adaptation to abiotic stresses such as freezing and drought (Arndt et al. 2001; Livingston III et al. 2009). These compounds help to stabilize cell membranes (Hincha et al. 2007), to prevent cell volume reduction by increasing the osmotic pressure, and to depress the freezing point (Krasensky and Jonak 2012). Consistent with this, fructans-accumulating plants are especially abundant in temperate and arid climate zones with seasonal frost or drought periods, and are almost absent in tropical regions (Hendry 1993b). Thus, we could expect starch hydrolysis to produce these compounds in both low and high elevation plants, but at different times. In low elevation, high low molecular weight sugars concentration would occur in mid-summer, while at higher elevations, it would occur in early autumn.

In this study we characterized the seasonal dynamics of carbon gain, growth and NSC concentration in an alpine plant species of the Andes of central Chile at two contrasting elevations. We hypothesize that the seasonal dynamics of growth and NSC concentrations in alpine plants of Mediterranean biomes is co-controlled by temperature and soil moisture. We expect that whilst in low elevation carbon gain is greatest at late-spring and early-summer, at high elevation the maximum carbon gain occurs during mid- and late- summer. We further expect that the end of the growing season coincides with the occurrence of severe drought in the lower elevation, and with the temperature decrease of autumn at the higher elevations. Thus, we anticipate that the highest NSC concentration occurs at autumn in the high elevations and at mid-summer in the low elevations. Finally, we anticipated that pervasive summer drought in the low elevations will induce elevational differences in the carbohydrate composition, with a greater fraction of osmotically active carbohydrates (i.e. fructans, sugars) during the driest period.

Materials and Methods

Study species

Phacelia secunda J.F. Gmel. (Boraginaceae) is a perennial hemicryptophyte herb with a wide elevational and latitudinal distribution in Chile and Argentina.

In Chile, it is distributed from ca. sea level to the upper vegetation limit in the Andes, inhabiting coastal, Mediterranean-type, montane forest and alpine communities (Cavieres 2000). Our study area was located in the Andes of central Chile, where *P. secunda* is distributed between 1600 and 3600 m a.s.l., showing clinal morphological variations along the elevational gradient (Cavieres 2000). At lower elevations, *P. secunda* enters dormancy immediately after fructification, while at high-elevations, vegetative growth continues throughout the summer and early fall, after the reproductive period (Arroyo et al. 1981).

Study site

Photosynthetic and growth measurements and plant tissues collection for NSC determination were carried out at two elevations in the Andes of central Chile, where there is a strong influence of the Mediterranean-type climate that particularly affects the low elevations (Cavieres et al. 2006). The low-elevation site was selected at 1600 m a.s.l. in the private natural reserve Yerba Loca (33°10’S, 70°13’W). At this elevation, the period free of snow lasts 8 months, usually starting in September and ending in April. The historical mean annual precipitation at this elevation is 445 mm, falling predominantly as rain during winter months (Santibañez and Uribe 1990). Growing season’s mean temperature at this elevation is 13.1 °C, with minimal and maximal temperatures of 10.6 and 22.5 °C, respectively (Hernández-Fuentes et al. 2015). The high-elevation site was selected at 3600 m a. s. l. (31°19’S, 70°15’W). At this elevation, the growing season lasts ca. 5 months (from

December to April), and the annual precipitation is 943 mm, falling mainly as snow during the winter with occasional hail or snow during the summer (Santibañez and Uribe 1990). Thus, in contrast to the low-elevation's site, this site has no water limitations throughout the growing season (Sierra-Almeida and Cavieres 2010). Growing season's mean temperature at this elevation is 8.2 °C with minimal and maximal temperatures of –1.0 and 20.1 °C, respectively (Hernández-Fuentes et al. 2015).

Sampling design

We collected plant tissues for NSC analyses during two consecutive growing seasons (2016- 2017 and 2017- 2018). The growing seasons were defined from October to April at the low- elevation (1600 m) and from December to April at the high- elevation (3600 m). The beginning of the growing season at each elevation was determined by the snowmelting. Seven similar-size and visually healthy individuals were monthly sampled at each site. Plants were carefully dug up, attempting to extract the complete root system. However, due to the large radical system of *P. secunda*, it was not possible to harvest whole roots. Immediately after collection, plants were placed in paper bags, properly labelled, and microwaved for 3 cycles of 20 seconds each to stop enzymatic activity. The day after collection, plants were transported to the Laboratory of Cultivo de Tejidos Vegetales (University of Concepción, Concepción, Chile).

To further characterize the soil water availability in our study sites, we measured the soil volumetric water content at each elevation and during two growing seasons. ECH$_2$O S–SMA–M005 probes, connected to a

HOBO® Micro Station logger, were buried at −30 cm depth. We additionally measured air temperatures with a HOBO® Micro Station logger (Onset Computer Corporation). Readings were recorded every one hour, and the daily averages were calculated.

Photosynthesis

The photosynthetic capacity was measured monthly during the second growing season (2017-2018). We selected and labelled with flagging tape ten adult plants at 1600 m and 3600 m, in October 2017 and December 2017, respectively.

A gas exchange system (GFS-3000, Walz, Germany) was used to measure photosynthetic capacity (A_{max}) in fully developed young leaves of seven from the 10 selected plants. All measurements were taken monthly at midday. Environmental conditions in the chamber used for leaf measurements consisted of 1500 µmol m^{-2} s^{-1} of photosynthetic photon flux density of, 15 °C of air temperature, 400 ppm of ambient CO_2 concentration and ca. 50 % of relative humidity.

Growth measurements

In each of the ten plants selected for gas exchange measurements, growth was measured non-destructively as the monthly increment in leaf length, rosette diameter and the number of leaves. The rosette diameter of each plant was measured with a measuring tape in a north-south direction. The leaf length was measured in 5 selected fully expanded leaves per plant from the base of the petiole to the leaf tip. The five values obtained per plant were averaged to obtain a single leaf length value per individual and date. The number of leaves was recorded counting all green leaves. The leaves length, rosette diameter and leaves number increment were estimated as the $\log_{10}$ ratio as follow:

$LRR\_LL = \log_{10}(LLf/LLi)$,

$LRR\_RD = \log_{10}(RDf/RDi)$,

$LRR\_LN = \log_{10}(LNf/LNi)$,

where, LRR means log-response ratio, LL, RD and LN means leaf length, rosette diameter and leaves number, respectively. LLi, RDi and LNi correspond to initial values (first month of measurement) and LLf, RDf and LNf corresponds to the value after a given time. Values > 0 indicate LL, RD or LN increases; values < 0 indicate LL, RD or LN decreased.

Carbohydrate analyses

Non-structural carbohydrate (NSC) concentrations were determined for above and belowground tissues separately, as the sum of the quantitatively low molecular weight carbohydrates (*i.e.* glucose, fructose and sucrose, hereafter “total soluble sugars”: TSS) plus starch as described in Hoch et al. (2002). Low molecular weight sugars were extracted with distilled water and analysed photometrically at 340 nm on a 96-well multiplate reader after enzymatic conversion (invertase and phosphoglucose isomerase from *Saccharomyces* *cerevisiae*, Sigma Aldrich I4504 and P5381, respectively, St Louis, MO, USA) of sucrose and fructose to glucose. The concentration of glucose was determined photometrically after the enzymatic conversion of glucose to gluconate-6-phosphate (Glucose Assay Reagent, G3293 Sigma Aldrich). Following the degradation of starch to glucose using a purified fungal amylase (‘amiloglucosydase’ from *Aspergillus niger*, Sigma Aldrich 10115) at 45°C overnight, starch was determined in a separate analysis by the same procedure. The starch concentration was calculated as NSC minus the sum of low molecular weight carbohydrates and expressed as glucose equivalents. Concentrations are presented on a percent of dry matter basis (% d. m.).

To compare the NSC concentration between elevations, the mean NSC concentration for each plant was weighted, integrating the biomass and NSC concentration of each tissue, as described in Hoch et al. (2002), using the following formula:

$$\sum_{org=1}^{n} \frac{conc_{org}\ x\ biom_{org}}{100},$$

where n is the number of organs, $conc_{org}$ is the organ-specific NSC concentration (% d. m.) and $biom_{org}$ is the organ-specific fraction of the total biomass. For roots, we used the biomass of the organ that was collected, which we acknowledge represents a fraction of the total. Nevertheless, we assume that the lost fraction was a similar proportion of the biomass in all individuals. On the other hand, we estimated the ratio between TSS and the total NSC concentration.

We also determined fructans concentrations, which are major forms of carbohydrate storage in alpine plants (Körner 2003a; Tolsma et al. 2008; Wyka 1999). Fructans concentration was measured using the Megazyme Fructans HK Assay kit (AOAC Method 999.03 and AACC Method 32.32; Megazyme International Ireland Ltd., Wicklow, Ireland), following the procedure described by McCleary et al. (2000). Fructans were extracted by digesting 100 milligrams of sample in 40 ml of distilled water at 80°C during 15 min. Aliquots of extract were treated with sucrose/amylase (sucrase/amylase mixture; bottle 1 in Megazyme Fructans assay kit A98YV29, plus sodium maleate buffer at 100 mM, pH: 6) to hydrolyze sucrose to glucose and fructose and starch to glucose. In one of the previous aliquots, fructans were hydrolyzed to fructose and glucose with purified fructanase (fructanase; bottle 2 in Megazyme Fructans assay kit A98YV29, plus sodium acetate buffer at 100 mM, pH: 4.5). Sugar's concentration was measured at 410 nm with the para-hydroxybenzoic acid hydrazide (PAHBAH) method for reducing-sugars and subsequently compared. Fructans concentration was determined as the difference in sugars concentration between the aliquot treated with fructanase and aliquots that were untreated.

To determine the fructans concentration, the following formula was used, as indicated in the kit: $\Delta A \times F \times \frac{V}{W} \times 2.48$ where, ΔA is absorbance of the extract treated with fructanase minus the absorbance of the blank, F is fructose factor (54.5μg / absorbance of fructans), V is extraction volume, W is sample weight, and 2.48 corresponds to correction for all solutions during procedure.

The whole-plant mean fructans concentration was estimated for each plant using the same procedure to estimate whole-plant NSC concentration.

Statistical analyses

To analyze the dynamics of photosynthesis and growth we used repeated measurements lineal models where photosynthesis, LRR_LL, LRR_RD and LRR_LN were the response variables, and elevation, time (months) and interaction between them were the explanatory variables. The seasonal dynamics of NSC concentration and its components (starch, fructans, and TSS) were analyzed with a similar model, where the concentrations were the response variables and elevation, time (months), growing season (2016-2017, 2017-2018) and all their interactions were the explanatory variables. All analyses were performed using the ‘nlme’ package (Pinheiro et al. 2013) in R (R 3.6.0). Differences between elevation and dates were tested using packages ‘emmeans’ and ‘lsmean’ (Lenth 2016). Tukey honest significant difference test (HDS) was used to evaluate differences between means (alpha < 0.05).

Results

Microclimatic variables

Mean daily air temperatures during the growing season varied with elevation (Fig. 1.1a). The low elevation site (1600 m) showed higher mean daily temperatures (16 °C) than the high elevation site (3600 m; 10 °C). At the low elevation, October was the month with the lowest mean temperature, while the highest daily mean temperatures were observed from January to April. At the high elevation, the month with the lowest average temperature was April, while December was the month with the highest average temperature. Throughout the growing season, the soil volumetric water content also varied with elevation and decreased progressively throughout the growing season (Fig. 1.1b). On average, the low elevation site showed lower soil water content (0.141 m^3 / m^3) compared to the higher elevation site (0.289 m^3 / m^3). At the low elevation, there was a strong decrease in soil water content in January, which was not observed at the high elevation.

Photosynthesis and stomatal conductance

In both elevations, the photosynthetic capacity varied significantly during the growing season (Fig. 1.2). Nevertheless, this seasonal dynamic significantly differed between elevations (significant effect of “elevation * time” on photosynthesis, Table 1.1). Plants at 1600 m showed a progressive decrease in photosynthesis from November to April; at mid-summer photosynthesis was only 20% of the maximum photosynthesis reached during the growing season, which was observed in October and November (Fig. 1.2). Plants at 3600 m, by contrast, showed an increase in photosynthesis from the beginning of the growing season in December to mid-summer in February (HSD Tukey test $p < 0.001$), and then a progressive decrease to the end of the season (HSD Tukey test $p < 0.03$; Fig. 1.2).

In both elevations, stomatal conductance varied significantly during the growing season (Fig. 1.2). Nevertheless, this seasonal dynamic significantly differed between elevations (significant effect of “elevation * time” on stomatal conductance, Table 1.1). Plants from 1600 m showed a progressive decrease in stomatal conductance from November to April (Fig. 1.2). Plants at 3600 m, by contrast, showed an increase in stomatal conductance from the beginning of the growing season in December to mid-summer (February) (HSD Tukey test $p < 0.001$), and then a progressive decrease to the end of the season (HSD Tukey test $p < 0.001$; Fig. 1.2).

Growth dynamic

Elevation, time and their interaction had a significant effect on the number of leaves (LRR_LN), leaf length (LRR_LL), and rosette diameter (LRR_RD) (Table 1.2). Plants at 1600 m increased the number of leaves progressively from the beginning of the growing season (October) to December (HSD Tukey test $p < 0.001$), and then remaining unchanged until February. Then the number of leaves decreased significantly until April (February: April, HSD Tukey test $p < 0.001$) (Fig. 1.3a). Plants at 3600 m increased the number of leaves from the beginning of the growing season (December) to February (HSD Tukey test $p < 0.001$) and showed no change afterwards (HSD Tukey test $p < 0.054$) (Fig. 1.3a). Leaf length increased through the growing season in both elevations, but the maximum increases and thus the maximum lengths were observed earlier at the lower elevation (Table 1.2). Plants at 1600 m rapidly increased the leaf length from October to December (HSD Tukey test $p < 0.001$) and showed no change afterwards (HSD Tukey test $p = 0.826$). Plants at 3600 m showed a significant increase in leaf length from December to February (HSD Tukey test $p < 0.001$) and showed no change afterwards (HSD Tukey test $p = 0.924$) (Fig. 1.3b). Plants at both elevations increased rosette diameter since the beginning of the growing season until December (HSD Tukey test $p < 0.001$), showing no change afterwards (HSD Tukey test $p = 0.935$ and $p = 0.726$).

NSC concentrations dynamics

In both elevations, NSC concentration in *P. secunda* varied during the growing season, and these variations were similar between the two growing seasons (significant effect of “time” on NSC concentration and non-significant “E*GS interaction, Table 1.3 and Fig. 1.4). Mean NSC concentrations throughout the growing season were significantly higher in plants from 3600 m than in plants from 1600 m (Table 1.3, Fig. 1.4). NSC concentration dynamic differed between elevations (significant effect of “elevation* time” on NSC concentration, Table 1.3 and Fig. 1.4), with maximum NSC concentration reached in February and April at 1600 m and 3600 m, respectively. Minimum NSC concentrations were found between October and December at 1600 m elevation and between November and January at 3600 m elevation (Fig. 1.4). Specifically, NSC concentration did not change for the first 3 months (October-November-December; HSD Tukey test: $p > 0.05$ for all combinations) in plants at 1600 m. After December, NSC concentration increased significantly until February (December-February, HSD Tukey test: $p < 0.001$ for both) in both growing seasons. Between February and April, NSC concentrations did not vary (February-March-April; HSD Tukey test: $p > 0.05$ for all combinations) for the 2016-2017's growing season but significantly decreased (ca. 10% from February to April) for the 2017-2018's growing season (February-April; HSD Tukey test: $p < 0.017$). At 3600 m elevation's plants, NSC concentration did not change from November to January in any of the two growing seasons (November-December-January; HSD Tukey test: $p > 0.05$ for all combinations). After January, NSC concentrations increased significantly and progressively until April. This increase was 2.8- and 2.2-fold, relative to the minimum NSC concentrations, during first and second growing season, respectively (Fig. 1.4a and b).

Dynamics of starch, fructans and totals soluble sugar

Elevation had a significant effect on starch, fructans and TSS concentration (significant effect of “elevation” on all carbon compound concentrations, Table 1.4). Plants at 3600 m presented, on average, greater starch, fructans and TSS whole-plant concentrations than plants at 1600 m (Fig. 1.5). Likewise, starch, fructans and TSS concentrations were affected by time (significant effect of “time” on all compound concentrations, Table 1.4). However, this effect varied with elevation (significant effect of “elevation” * “time” on all compound concentrations, Table 1.4). Plants at 1600 m showed an increase in TSS concentration between October and January (HSD Tukey test $p < 0.001$) and then TSS remained unchanged until April during both growing seasons (Fig. 1.5a-b). At 3600 m, and for the first growing season only, plants showed a slight decrease in TSS concentrations from November to December (HSD Tukey test: p <0.001) and an increase from December to April (Fig. 1.5a). During the second growing season, plants at 3600 m increased its TSS concentration from November to April (HSD Tukey test: p <0.001). The magnitude of the increase observed in plants at 3600 m was smaller than that observed in plants from 1600 m.

In both growing seasons, starch concentration in plants at 1600 m elevation (Fig. 1.5c) showed a slight increase from October to January (HSD Tukey test p <0.001) and then a sharp increase from January to February (HSD Tukey test p <0.001) (Fig. 1.5c-d). After February of the first growing season, starch in plants at 1600 m remained at a same concentration until the end of the growing season. However, a 10% decrease in starch was found between February and April (February-April; HSD Tukey test: p < 0.017) in plants at 1600 m during the second growing season (Fig. 1.5d). In plants at 3600 m, starch concentration increased sharply from January to February. However, in contrast to plants from 1600 m, starch concentration continued increasing until April (Fig. 1.5c-d).

Fructans' concentration decreased from October to January (HSD Tukey test p <0.001) and increased afterwards (HSD Tukey test, p<0.001) for both growing seasons at 1600 m (Fig. 1.5e-f). At 3600 m, fructans' concentrations between November and December increased during the first season (HSD Tukey test, p<0.001), but they decreased during the second growing season (HSD Tukey test, p<0.001). Fructans' concentrations then decreased strongly until February during the first growing season (Fig. 1.6e). In both growing seasons, concentrations at 3600 m increased to March and decreased afterwards (HSD Tukey test, p<0.001) (Fig. 1.6f).

Totals soluble sugar: Nonstructural carbohydrates ratio (TSS: NSC)

Elevation had a significant effect on TSS: NSC ratio (significant effect of "elevation", Table 1.5). However, differences between elevations were time dependent (significant effect of "elevation" * "time" on TSS: NSC ratio, Table 1.4). Plants at 1600 m showed an increase in TSS: NSC ratio from October to January (HSD Tukey test p <0.001) and then a significant decrease from January to April (HSD Tukey test p <0.001), during both growing seasons (Fig. 1.6). In plants at 3600 m, TSS: NSC ratio had a significant decrease between December and January (HSD Tukey test p <0.001) and was then maintained at very low levels until April (HSD Tukey test p = 0.100) during both growing seasons (Fig. 1.6).

Discussion

We found that the seasonal dynamics of photosynthesis, growth, and carbohydrate concentrations in the widely distributed alpine plant species *Phacelia secunda* differed significantly between two contrasting elevations. The differences we found can be hardly explained by temperature alone. Rather, they are consistent with a role of drought controlling the timing and length of the growing season at low elevation. Thus, the highest photosynthetic rate at 1600 m occurred by mid-spring (Fig. 1.2), coinciding with the period of higher soil moisture (Fig. 1.1). Then, photosynthesis decreased progressively at this elevation along with the progression of summer drought and the reduction in stomatal conductance, something that has been previously reported in *P. secunda* at low elevation (Hernández-Fuentes et al. 2015). The faster decrease of stomatal conductance compared to photosynthesis from November to January at 1600 m, which does not occur in plants of 3600 m, indicates a greater water use efficiency (WUE) for 1600 m plants. Increased WUE is a well-known plant response to drought associated with drought resistance (Piper et al. 2007). Thus, in plants of the lower elevation, greater WUE could be a mechanism conferring greater resistance to drought, in comparison with plants of higher elevation. Additionally, the maximum plant growth and NSC concentrations were observed ca. 2 months earlier at 1600 m than at 3600 m (Figs. 1.3 and 1.4). Maximum NSC concentration found towards the end of the growing season, as we found at 3600 m, is consistent with the patterns reported in most alpine plants (e.g. Hiltbrunner et al. 2021). Maximum NSC concentrations at 1600 m (achieved ca. 2 months earlier than at 3600 m) occurred before summer drought took place, suggesting that summer drought restricted further increases in NSC concentration. Most likely, drought contributed to the C reserves remobilization by late summer, indicated by the significant decrease in starch concentrations during the late summer of the second growing season. Hence, although the altitudinal temperature decrease is a worldwide major driver of the phenology and carbon balance in alpine plants (Körner 2003a; Mooney and Billings 1965; Schappi and Korner 1997), our study shows that drought significantly alters the growth phenology and the NSC seasonal dynamics.

In both elevations, C reserves exhibited minimal levels during the growing period and maximum levels coinciding with the end of growth. This pattern confirms the importance of C reserves in alpine plants to support the high C demands associated with growth reactivation in spring (Mooney and Billings 1960). Contrary to our prediction, low elevation plants stopped growth during late spring (not mid-summer) along with a decrease in photosynthesis and stomatal conductance, all of which were likely reduced by a significant decrease in the soil water content (Fig. 1.1b). At this elevation, therefore, temperature and soil moisture conditions are favorable for growth only during early spring, and these favorable conditions last for a couple of months before the onset of drought (Peñaloza et al. 2001). These plants showed a quick gain in biomass during spring. Then, when growth stopped, the NSC increased 3-times. Transitory NSC increases typically result from photosynthesis being less sensitive to drought than growth (Boyer 1970; Muller et al. 2011). Thus, the increase in NSC concentrations observed until mid-summer was most likely the result of the cessation of growth along with the reduced but still significant photosynthetic capacity. However, a smooth but significant decrease in NSC and starch concentrations was observed at the lower elevation for the late summer of the second growing season. Contrary, NSC and starch concentrations continued increasing by late summer and autumn at the higher elevation. This contrasting NSC and starch dynamics may be explained by the use of C reserves to support metabolic demands associated by summer drought at the lower elevation, where high temperatures along with reduced photosynthesis likely implied a negative C balance (e.g. Adams et al. 2013). By contrast, continued increase of carbohydrates at the higher elevation was promoted by constantly high photosynthetic rates and, probably, lower metabolic costs associated with the lower temperatures.

Mean NSC concentrations during the whole growing season were greater at 3600 m than at 1600 m. At the beginning of the growing season, NSC concentrations were 2.4 times higher at 3600 m than at 1600 m. Similarly, at the end of the growing season, plants at 3600 m showed 1.8 times higher concentration of NSC than plants at 1600 m. The higher concentration of NSC in high-elevation plants compared to low-elevation plants could be the result of a lower demand for C. Although plants from both elevations presented the same photosynthetic capacity, the higher-elevation plants are smaller, which could imply less use of C for biomass and a higher C surplus available for storage. On the other hand, this result could reflect a prioritization of C allocation to storage over growth (Wiley and Helliker 2012). It has been previously reported that, after severe C limitation, *P. secunda* plants from 3600 m favors more strongly C allocation to storage than to growth compared to plants from 1600 m (Reyes-Bahamonde et al. 2021). Such high NSC concentrations could be advantageous to persist under environmental conditions that can cause tissue damage or removal (summer frosts, snowpack) and hence C limitation and C starvation. It has been postulated that plant persistence in stressful environments is favored by C allocation to storage (Grime 2006; Wiley and Helliker 2012). Indeed, the short growing season of alpine habitats is often characterized by the occurrence of unpredictable disturbances which may constrain the C gain or destroy the photosynthetic tissue (e.g. days with low light irradiance, summer snow, late-spring or summer frosts) (Körner 2003a). For example, the long duration of the snow cover period characterizing higher elevations forces plants to survive at the expense of their C reserves, and often to start budbreak and growth under snow. The NSC concentration at the end of the growing season could determine the survival of *P. secunda* plants from high elevations during the snow period. In congruence with this idea, Körner et al. (2019) showed that the alpine plant *Soldanella pusilla* grew during the winter under 2-3 m of snow thanks to the large concentrations of NSC that this species accumulated during the summer. In contrast to high elevation plants, our results suggest that the period of reliance on carbon reserves for early growth is shorter for low elevation plants, as the highest photosynthetic capacity is achieved early in the season, potentially conferring a fast C autonomy. This may also explain why these plants enter to winter accounting for lower NSC concentrations than high elevation plants, and why low elevation plants did not show constantly high NSC concentrations (relative to seasonal maximums) as high elevation plants did. Our results also show elevational differences in the dynamics of NSC fractions. At the low elevation, the dynamics of TSS and fructans were consistent with the presence of drought since January on. Thus, in both growing seasons, TSS concentrations increased from December to January (although only significantly in the second growing season) and remained at this concentration until the end of the growing season. Although starch also increased from spring to early summer, it then decreased (only significantly during the second growing season). These results are consistent with a peak in TSS concentrations around midsummer reported for plants inhabiting Mediterranean ecosystems (Martínez-Vilalta et al. 2016) (. These results suggest that, for low elevation plants, to maintain high TSS concentrations during the dry period may be critical. Low molecular weight sugars have osmotic activity, and their concentrations typically increase in response to drought (Ahmad et al. 2020; Arndt et al. 2001). The fact that the increase in TSS concentrations was more noticeable in the second growing season could be explained by the greater severity of drought at this growing season regarding to the first one (Online Resource 3). Increasing concentrations in fructans since January on could be also related drought, as fructans have been also found to have osmotic activity during drought stress (Garcia et al. 2011). On the other hand, the results observed in plants at 3600 m are consistent with the exposure of plants at low temperatures. At this elevation, plants showed that both TSS and fructans increased to mid-autumn, when temperatures decreased. Similarly, Payton and Brasch (1978) found that both starch and TSS concentrations of two alpine plant species (*Chionochloa rigida* and *C. macra*.) in New Zealand reached their maximum towards the beginning of autumn. Our results suggest that in plants of this elevation fructans as well as TSS could play a cryoprotective role, as reported previously for plants exposed to low temperatures (Livingston et al. 2009). The strong seasonal variation in the fructan dynamic was markedly different between the two elevations. The most pronounced seasonal changes at 3600 m than at 1600 m suggest that temperature was a more important driver of the fructan dynamics than soil moisture. Fructans are known to protect the integrity of membranes and cellular organelles as well as the photosynthetic machinery, and to maintain the redox potential (Abeynayake et al. 2015; Demel et al. 1998). The fact that the fructan concentration peak occurred after the starch concentration peak in both seasons, suggests that starch synthesis is a priority relative to fructans synthesis. This suggests signify that plants prioritize the survival during winter and the spring regrowth (processes supported by starch) than the vitality of the current tissues. Although TSS concentrations increased towards the autumn in both elevations, the TSS: NSC ratio did not increase but rather it decreased, as starch concentrations were overall higher at the end than at the mid of the growing season. Therefore, our results indicate that the increase in osmotic requirements of *P. secunda*, associated with either drought or low temperature, occur at high levels of C reserves.

Conclusions

Our study showed that the temporal dynamic of growth phenology and C reserves of *P. secunda* differed significantly between plant populations at high and low elevations, and that this difference cannot be explained by the elevational decrease in temperature only. Rather, our results point to a significant role of drought shaping the seasonal dynamics of NSC concentrations and growth phenology of alpine plants of Mediterranean biomes. Although the elevational decrease in temperature constrains the growing season length, leading to longer duration of the growing season at lower elevations (Zachhuber and Lacher 1978), drought appears constraining the growing season length at the lower elevations. As a result, the duration of growing season was similar at contrasting elevations. Our results therefore highlight a role of drought, and not only of temperature, to predict responses of alpine plants to climate change in Mediterranean biomes. Mediterranean high-mountain ecosystems have been identified as especially susceptible to global warming (Lionello 2012), caused by the rise in average summer air temperature and the reduction in annual rainfall (Nogués-Bravo et al. 2012). Specifically, our study shows that in the alpine plant *P. secunda* inhabiting Mediterranean biomes, drought determines the length of the growing period and the dynamics of NSC at the end of this period. More severe droughts are thus expected to cause a shorter growing period along with more severe reductions in NSC concentrations, which could cause insufficient levels of C reserves to meet demands of wintertime and early spring. However, our study considered only one alpine species and only two sites, remaining uncertain whether our findings represent a general the responses of other Mediterranean alpine plants.

Declarations

Financial interests: The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Tables

Table 1.1 Results from repeated measures ANOVA testing the effects of elevation, time, and the interaction between them on photosynthetic capacity and stomatal conductance (Fig. 1.2).

Factor	d.f	F. ratio	P-value
Photosynthesis			
Elevation	1	27.48	<0.001
Time (T)	6	22.34	<0.001
E*T	5	80.12	<0.001
Stomatal conductance			
Elevation	1	1020.93	<0.001
Time (T)	6	122.50	<0.001
E*T	5	194.71	<0.001

d.f. stands for degrees of freedom

Table 1.2 Results from a general lineal model (GLM) testing the effects of elevation, time and the interaction between them on growth of *Phacelia secunda* at 1600 and 3600 m at the Mediterranean Andes of Central Chile. LRR_RD stands for rosette diameter log-response ratio, LRR_LL stands for leaf length log-response ratio and LRR_LN stands for leaves number log-response ratio.

Factor	**d.f**	**F. ratio**	**P-value**
LRR_RD			
Elevation (E)	1	0.370	0.014
Time(T)	6	12.485	<0.001
E*T	6	4.568	0.004
LRR_LL			
Elevation (E)	1	48.697	<0.001
Time(T)	6	76.458	<0.001
E*T	6	6.547	<0.001
LRR_NL			
Elevation (E)	1	4702.095	<0.001
Time(T)	6	51.538	<0.001
E*T	6	3.012	0.0004

d.f. stands for degrees of freedom

Table 1.3 Results from a lineal model (LM) testing the effects of elevation, time, growing season and the interactions amongst them on NSC concentration (Fig.1.4)

Factor	d.f	F. ratio	P-value
NSC concentration (%DM)			
Elevation (E)	1	245.15	<0.001
Growing season (GS)	1	30.45	<0.001
Time(T)	6	115.75	<0.001
E * GS	1	0.25	0.534
E * T	6	9.48	<0.001
T * GS	6	2.68	<0.001
E * T * GS	6	0.074	0.742

d.f. stands for degrees of freedom

Table 1.4 Results from lineal models (LM) testing the effects of elevation, time and the interactions amongst them on concentrations of starch, fructans and soluble sugar (Fig. 1.5)

Factor	d.f	F. ratio	P-value
Starch (%DM)			
Elevation (E)	1	6.12	<0.001
Growing season (GS)	1	8.17	<0.001
Time(T)	6	104.83	<0.001
E * GS	1	0.24	0.645
E * T	6	2.87	<0.001
T * GS	6	2.37	0.027
E * T * GS	6	0.85	0.640
Fructans (%DM)			
Elevation (E)	1	2853.24	<0.001
Growing season (GS)	1	0.15	0.0626
Time(T)	6	145.45	<0.001
E * GS	1	8.06	0.001
E * T	6	52.12	<0.001
T * GS	6	24.24	<0.001
E * T * GS	6	14.15	<0.001
Soluble sugars (%DM)			
Elevation (E)	1	119.17	<0.001
Growing season (GS)	1	31.54	<0.001
Time(T)	6	15.45	<0.001
E * GS	1	0.28	0.685
E * T	6	7.95	<0.001
T * GS	6	1.85	0.145
E * T * GS	6	2.85	0.032

d.f. stands for degrees of freedom

Table 1.5 Results from lineal models (LM) testing the effects of elevation, time, and the interactions amongst them on TSS: NSC ratio (Fig. 1.6)

Factor	d.f	F. ratio	P-value
Elevation (E)	1	17.2401	5.40e-05
Time(T)	6	24.872	2.2e-16
Growing season (GS)	1	10.135	0.002
E*T	5	2.555	0.297
E*GS	1	0.034	0.854
GS*T	6	2.983	0.009
E*T*GS	5	1.672	0.145

d.f. stands for degrees of freedom

Figures

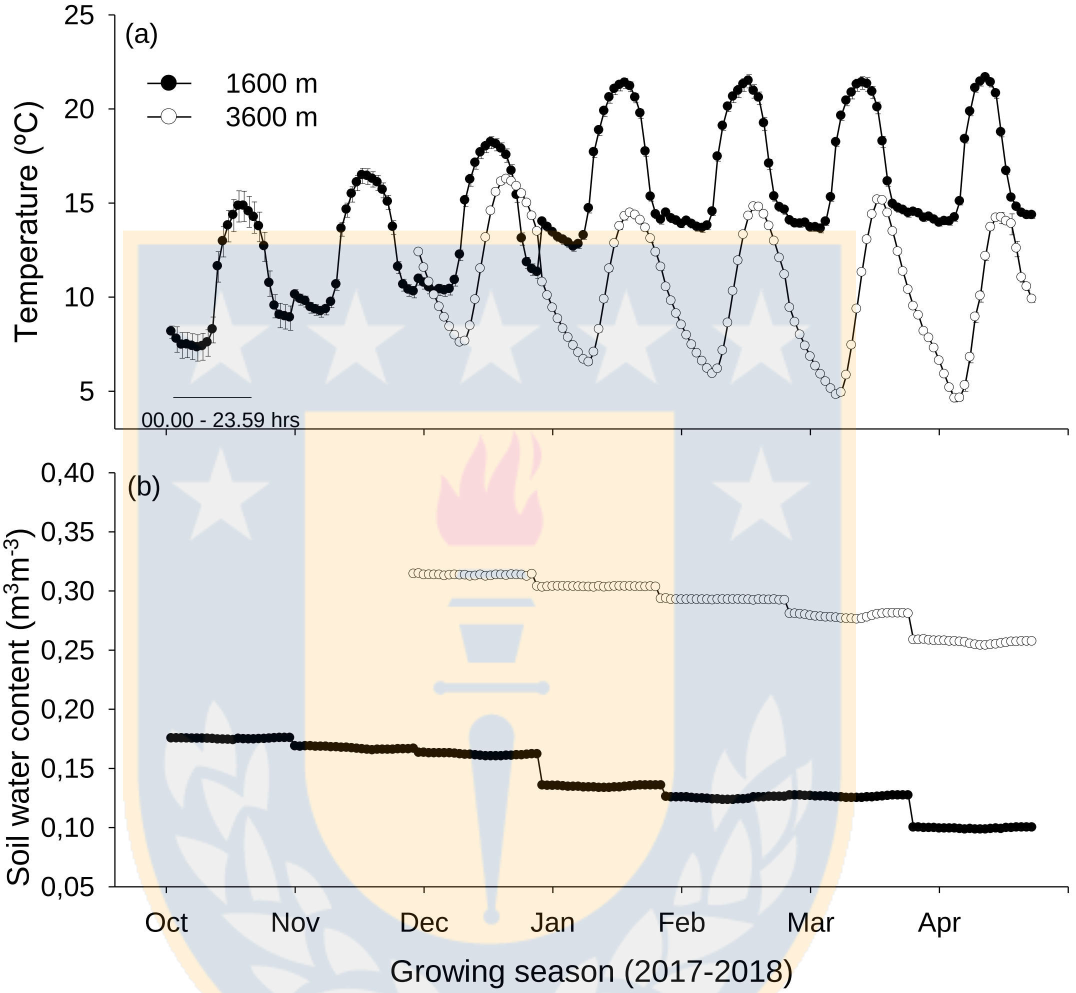


Figure 1.1 Daily average (a) air temperature and (b) soil water content registered at 1600 and 3600 m a.s.l. in the Andes of central Chile. Averages were calculated for two growing seasons (October 2016- April 2017 and October 2017- April 2018 for 1600 m site; December 2016- April 2017 and December 2017- April for 3600 m site).

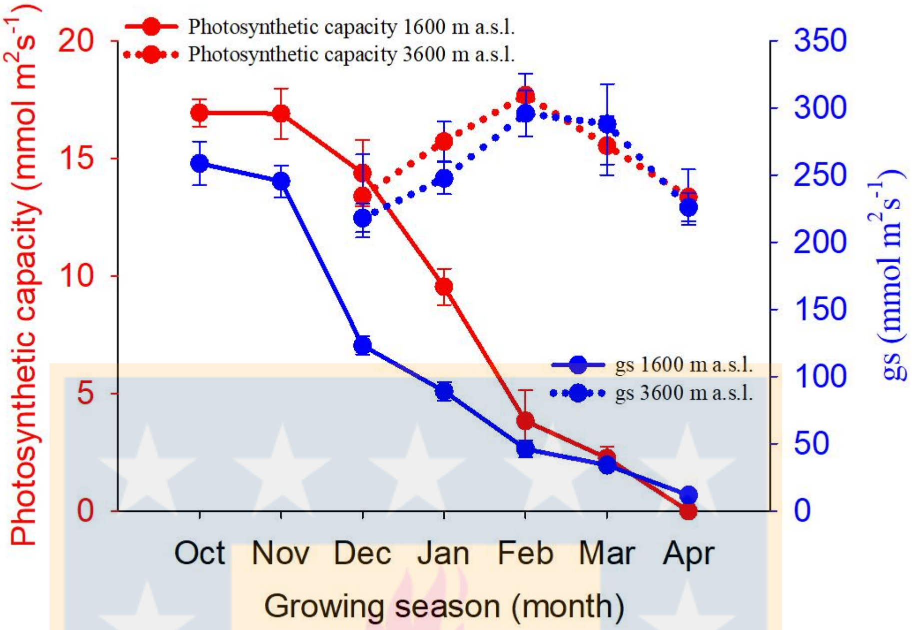


Figure 1.2 Seasonal dynamic of photosynthetic capacity and stomatal conductance (gs) in *Phacelia secunda* (mean ± SE) at 1600 m and 3600 m in the Mediterranean Andes of Central Chile during the second growing season of the study (2017- 2018).

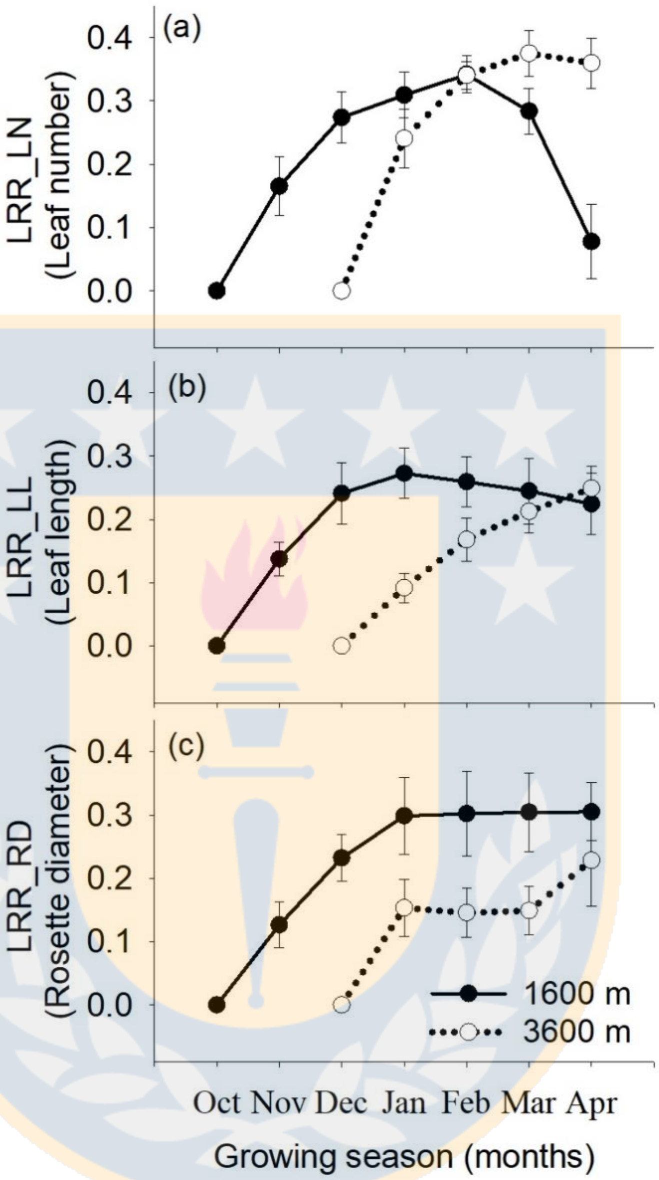


Figure 1.3 Seasonal dynamic of growth (mean ± SE) in *Phacelia secunda* at 1600 and 3600 m in the Mediterranean Andes of Central Chile during the second growing season of the study (October 2017- April 2018). (a) Log10 of the ratio between final and initial leaves number, (b) Log10 of the ratio between final and initial leaf length and (c) Log10 of the ratio between final and initial rosette diameter.

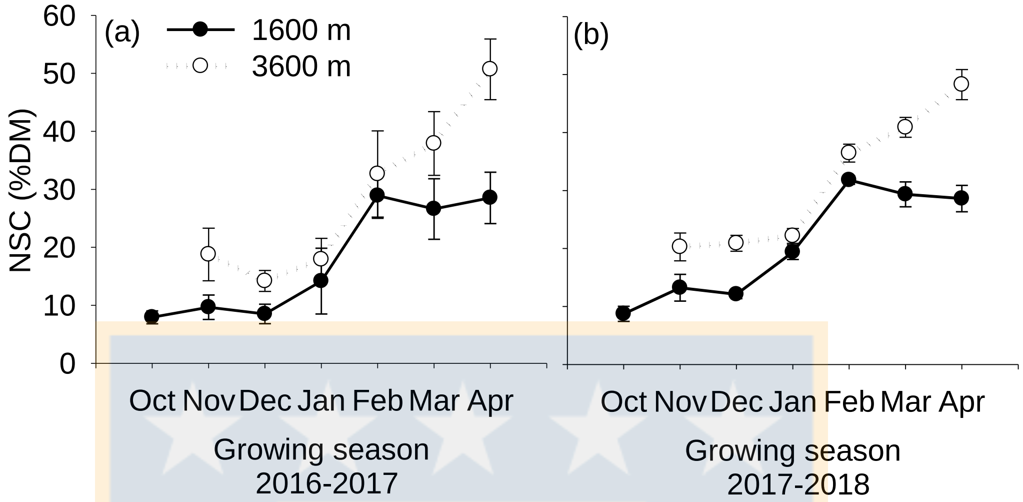


Figure 1.4 Dynamics of non-structural carbohydrates (NSC) concentrations (mean ± SE) for two consecutive growth seasons (October 2016- April 2017 and October 2017- April 2018 for 1600 m site; December 2016- April 2017 and December 2017- April for 3600 m site) in the alpine plant species *Phacelia secunda* naturally established at 1600 and 3600 m a.s.l. in the Mediterranean Andes of Central Chile.

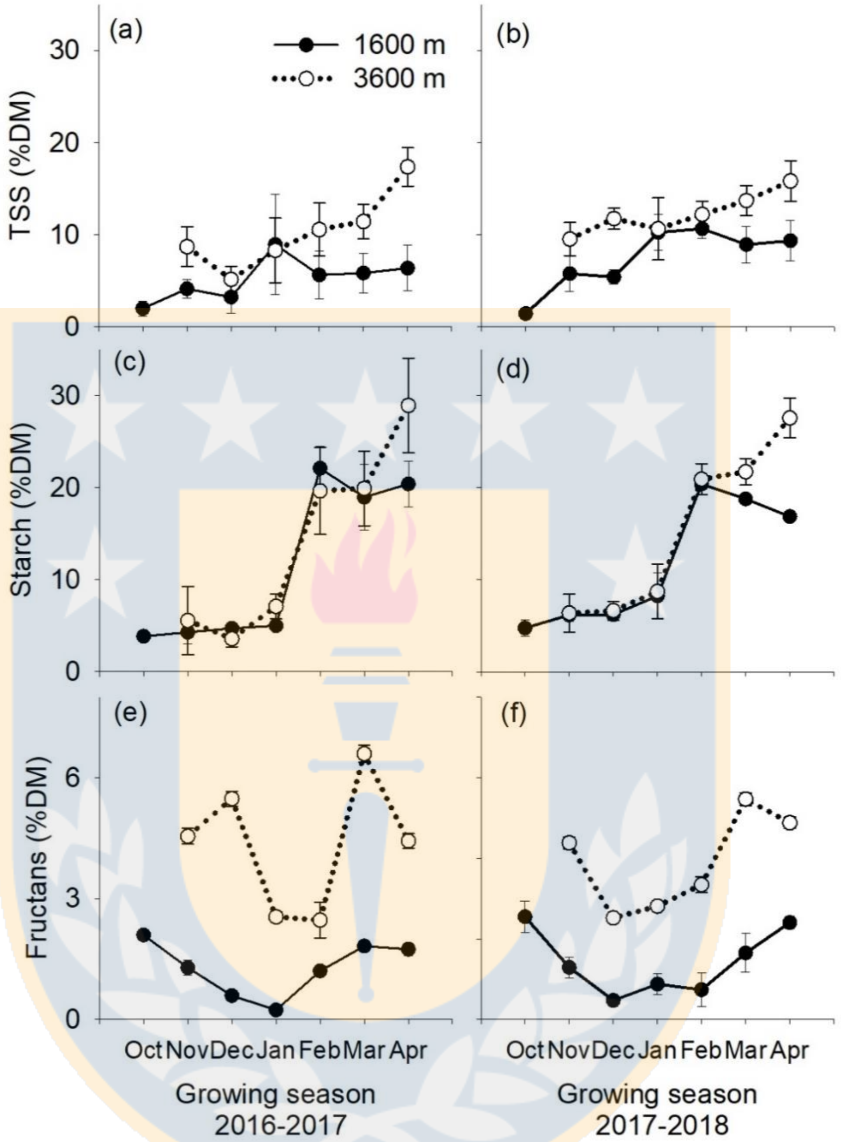
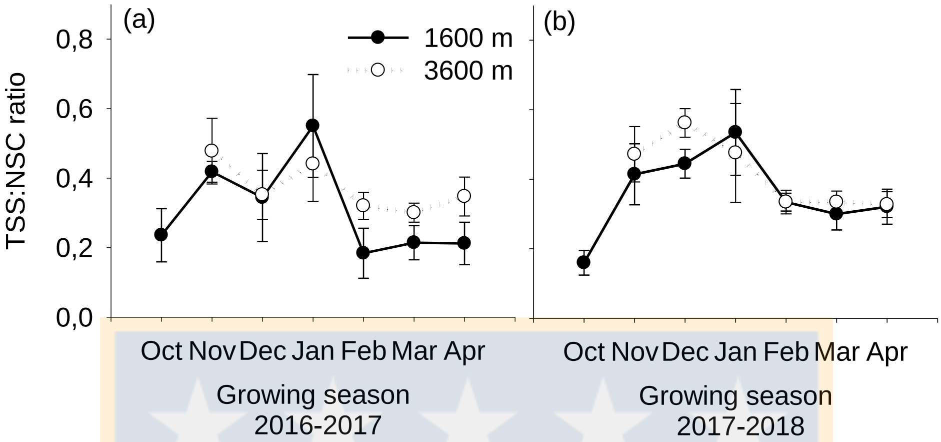


Figure 1.5 Dynamics of concentrations of starch, fructans and total soluble sugars concentrations (mean ± SE) for two consecutive growth seasons (October 2016- April 2017 and October 2017- April 2018 for 1600 m site; December 2016- April 2017 and December 2017- April for 3600 m site) in *Phacelia secunda* at 1600 and 3600 m in the Mediterranean Andes of Central Chile.



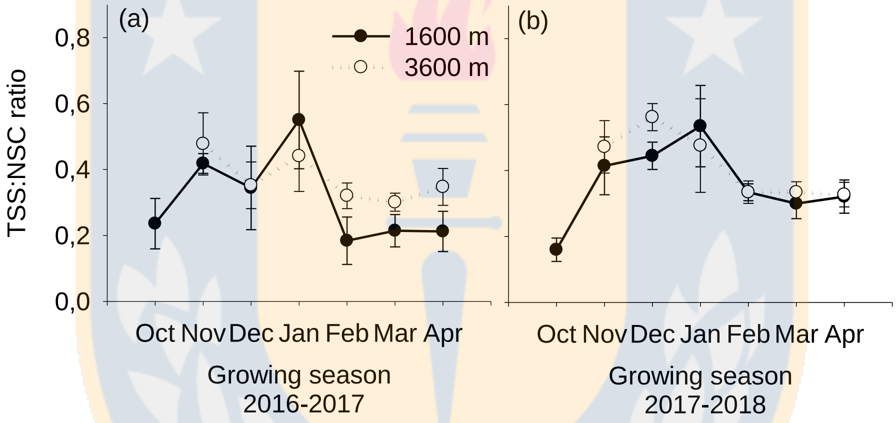


Figure 1.6 Total soluble sugars: Nonstructural carbohydrates (TSS: NSC) ratio (mean ± SE) for two consecutive growth seasons (October 2016- April 2017 and October 2017- April 2018 for 1600 m site; December 2016- April 2017 and December 2017- April for 3600 m site) in *Phacelia secunda* plants at 1600 and 3600 m in the Mediterranean Andes of Central Chile

CAPÍTULO II CARBON ALLOCATION TO GROWTH AND STORAGE DEPENDS ON ELEVATION PROVENANCE IN AN HERBACEOUS ALPINE PLANT OF MEDITERRANEAN CLIMATE

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Abstract

It is unclear whether the frequently observed increase of nonstructural carbohydrates (NSC) in plants exposed to low temperatures and drought reflects a higher sensitivity of growth than photosynthesis in such conditions (i.e. sink limitation), or a prioritization of carbon (C) allocation to storage. Alpine areas in Mediterranean-type climate regions are characterized by precipitation increases and temperature decreases with elevation. Thus, alpine plants with wide elevational ranges in Mediterranean regions may be good models to examine these alternative hypotheses. We evaluated storage and growth during experimental darkness and re-illumination in individuals of the alpine plant *Phacelia secunda* from three elevations in the Andes of central Chile. We hypothesized that storage is prioritized regarding growth in plants of both low- and high-elevations where drought and cold stress are greatest, respectively. We expected that decreases in NSC concentrations during darkness should be minimal and, more importantly, increases in NSC after re-illumination should be higher than increases in biomass. We found that darkness caused a significant decrease in NSC concentrations of both low- and high-elevation plants, but the magnitude of the decrease was lower in the latter. Re-illumination caused higher increase in NSC concentration than in biomass in both low- and high elevation plants (1.5- and 1.9-fold, respectively). Our study shows that C allocation in *Phacelia secunda* reflects ecotypic differences among elevation provenances and suggests that low temperature, but not drought, favors C allocation to storage over growth after severe C limitation.

Key words: carbon limitation, high-mountain plants, ecotypes, drought, low temperature stress.

Introduction

Plants growing in habitats characterized by low temperatures or water shortages generally present high concentrations of non-structural carbohydrates (NSC) (e.g. Fajardo and Piper 2014; Hoch and Körner 2003; Hoch and Körner 2009; Piper and Fajardo 2016; Sala and Hoch 2009; Zhang et al. 2015). Such high NSC concentrations could reflect carbon (C) accumulation once growth demands are met (Chapin et al. 1990; Körner 2003a), since tissue formation (i.e. cell division, elongation, and differentiation) is more sensitive to low temperature and water shortages than photosynthesis (Fatichi et al. 2014; Hoch 2015; Körner 2015; Muller et al. 2011). In this case, tissue formation is directly restricted by temperature or water availability (i.e. sink limitation) before carbon availability becomes limiting due to reduced photosynthesis (i.e. C or source limitation) (Boyer 1970; Körner 2015; Muller et al. 2011). Alternatively, increases in NSC concentrations in response to drought or low temperatures could actually reflect a change in the C allocation priorities from growth to storage (Dietze et al. 2014; Sala et al. 2012; Wiley and Helliker 2012). This could be part of a preventative strategy against potential C starvation driven by long periods of reduced photosynthesis due to drought or low temperatures (Wiley and Helliker 2012), or because the minimum NSC levels required for plant survival increase under water shortage or low temperature conditions (Martínez-Vilalta et al. 2016; Sala et al. 2012). The distinction between these two putative causes of storage (i.e. simple accumulation or an allocation priority) is highly relevant to predict vegetation responses to climate change. If storage reflects an allocation priority, “reserves” (sensu Chapin et al. 1990) could compete with growth, making C a limiting resource for plant growth despite increased NSC concentrations (Dietze et al. 2014; Sala et al. 2012; Wiley and Helliker 2012). The occurrence of a trade-off between storage and growth in plants exposed to low temperatures and/or drought is however still uncertain.

A prioritization of C allocation to storage has been suggested as an adaptation to optimize survival in plants inhabiting stressful environments (Chapin et al. 1990; Sala et al. 2012; Smith et al. 2003; Wiley and Helliker 2012). Several studies support this suggestion. For example, Myers and Kitajima (2007) found that high levels of C storage increased the survival in seedlings of seven tree species of tropical forests when they experienced negative C balance imposed by herbivory and light reduction. Similarly, O’Brien et al. (2014) found that seedlings of tropical tree species experimentally enriched in NSCs showed greater drought survival, and maintained higher stem water potentials than non-enriched seedlings. Some studies have demonstrated that allocation to storage may be prioritized over allocation to growth suggesting a trade-off between these two sinks. For example, Smith and Stitt (2007) showed that a limited C availability (carbon starvation) due to longer nights caused a strong growth inhibition during the next day along with higher rates of starch synthesis, suggesting that the carbon supplied by photosynthesis was assigned to storage to ensure a sufficient C supply for the next night. Piper et al. (2015) found that NSC concentration was significantly higher and radial growth was significantly lower, in branches of *Nothofagus pumilio* trees that were completely defoliated the previous season compared to non-defoliated control trees. Similarly, Weber et al. (2018) found that in seedlings, growth declined more than NSC concentration after a dark treatment, and that following re-illumination NSC refilling started before growth resumption. Nevertheless, these previous studies are not conclusive about potential ecotypic differences in C allocation priorities. If C allocation to storage is prioritized relative to growth as a preventative strategy against C starvation episodes, then such allocation priority should be expected in plant species frequently exposed to freezing temperatures or drought (Wiley and Helliker 2012). Contrary to these expectations, Bachofen et al. (2018) found that seedlings of two conifer species from dry provenances had both more biomass and starch concentrations than their counterparts from more humid regions. However, the drought gradient considered in that study was small, remaining thus unclear whether these results reflect C allocation patterns in plant species distributed along wider drought gradients. Additionally, while most studies examining C allocation priorities have focused on woody plants, a prioritization of storage vs. growth could be more prevalent in herbaceous plants, as they have a higher proportion of living, metabolically active tissues; hence, decreases in NSC concentrations (which may put them at risk of C starvation) for a given C demand are likely larger (Martínez-Vilalta et al. 2016).

Alpine plants are characterized by low stature and high year-round NSC concentrations(Monson et al. 2006; Mooney and Billings 1960; Wyka 1999).

Environmental restrictions such as short growing seasons, unpredictable freezing events, and other natural hazards including hails, landslides and long periods of low solar radiation that characterize alpine environments (Körner 2003a), could lead to severe C limitations and potentially to C starvation. Further, alpine plants develop new foliage as soon as snow melts, relying on the resources stored during the previous growing season. Both early- and late-season freezing events cause an abrupt cessation of C gain due to foliar damage and therefore reduce the effective growing season length (Körner 2003a). Since these disturbances are often unpredictable in most alpine ecosystems, to rely only on current photosynthesis to fulfil C demands for different sinks (growth, respiration, reproduction, defence, etc.) could be risky. Thus, alpine plants could have a C conservative strategy, keeping high levels of C storage during the growing season and prioritizing C allocation to storage after a period of severe C limitation or C starvation. Consistent with this hypothesis, it has been observed that alpine plants maintain high NSC concentration at periods of maximum growth (García-Lino et al. 2017; Monson et al. 2006; Wyka 1999). Starch, low molecular-weight sugars, and fructans are the main NSC fractions observed in alpine plants under low temperature conditions (Chatterton et al. 1989; García-Lino et al. 2017; Guevara-Figueroa et al. 2015; Valluru and Van den Ende 2008).

Alpine plant species inhabiting mountains with Mediterranean-type climate also experience severe summer droughts (Cavieres et al. 1998). In these ecosystems, temperature decreases with elevation while precipitation increases (Cavieres et al. 2006; Piper et al. 2016), resulting in “cold and wet high elevation" versus "warm and dry low-elevations". For mid-elevation, neither drought nor temperature appears to be as extreme, although growth is not necessarily intermediate between the two elevations (Piper et al. 2016). Previous studies have demonstrated that both temperature and water availability affect C storage and growth of the dominant treeline species in Mediterranean regions (Fajardo et al. 2011; Piper et al. 2016). Thus, alpine plants of Mediterranean regions could exhibit elevational differences in the prioritization of C allocation to storage versus growth, reflecting different selective pressures from drought and low temperature at lower and high-elevations, respectively.

Here we aimed to determine whether C allocation to growth and storage in the alpine plant *Phacelia secunda* (Boragineaceae) varies along an elevational gradient in the Mediterranean Andes of central Chile. We experimentally evaluated the effects of darkness-induced C limitation and subsequent re-illumination on the growth and C storage levels of *P. secunda* from three contrasting elevations. Although growth and storage responses during re-illumination after a darkness period are likely to reflect C allocation priorities after extreme C stress, NSC changes during darkness might also be informative on C allocation priorities. Darkness provokes a negative C balance and plants have to remobilize their C reserves for survival during this period. However, the magnitude of C reserves remobilization during C limitation periods depends on the metabolic demands, with fast-growing plants expected to remobilize proportionally more C reserves given to their higher metabolic demands when compared to slow-growing plants (e.g. Nogués et al. 2014;

Weber et al. 2018). Therefore, if C allocation to growth is a priority relative to storage, i) storage should not be maintained under darkness but remobilized and ii) re-illumination should cause higher increases in structural biomass than in NSC concentrations (Fig. 2.1a). By contrast, if storage is a priority relative to growth, we expect that iii) NSC concentrations would be maintained, and that iv) re-illumination will cause higher increases in NSC concentrations than in structural biomass (Fig. 2.1b). Since plants from the high- and low-elevations inhabit stressful conditions of low temperatures and drought, respectively, which are often associated with C stress, we might expect that populations adapted to such conditions will tend to store rather than to grow fast following C starvation. Conversely, as the environmental conditions where mid-elevation plants occur are intermediate in terms of stress severity, mid-elevation plants may not store C after a period of severe C stress. We examined these predictions by determining the changes in biomass and in concentrations of total NSC, fructans, starch, and low molecular weight sugars, which are three major forms of carbohydrate storage in alpine plants (Körner 2003a; Tolsma et al. 2008; Wyka 1999).

Materials and Methods

Study species

Phacelia secunda J. F. Gmel. (Boraginaceae) is a perennial hemicryptophyte herb with a wide elevational and latitudinal distribution in Chile and Argentina. In Chile, it is distributed from c. sea level to the upper vegetation limit in the

Andes, inhabiting coastal, Mediterranean-type, montane forest and alpine communities (Cavieres et al. 2000). Our study area was located in the Andes of central Chile, where *P. secunda* is distributed between 1600 and 3600 m a.s.l., showing "clinal" morphological variations along the elevational gradient (Cavieres 2000 (Cavieres 2000). At lower elevations, *P. secunda* enters vegetative dormancy immediately after fructification, while at high-elevations, vegetative growth continues throughout the summer and early fall, after the reproductive period (Arroyo et al. 1981).

Study site

Plant material was collected from three elevations in the Andes of central Chile, where climate has a strong influence of the Mediterranean-type climate that prevails in the low elevations. The collection area at the low-elevation was at 1600 m a.s.l. in the private natural reserve Yerba Loca (33°10'S, 70°13'W). At this elevation, the snow-free period lasts eight months, usually starting in September and ending in April. However, most plant species stop growth during the summer drought (i.e. January–February). The historical mean annual precipitation at this elevation is 445 mm, falling predominantly as rain during winter months (Santibañez and Uribe 1990). Growing season mean temperature at this elevation is 13.1 °C, with minimal and maximal temperatures for this period of 10.6 and 22.5 °C, respectively (Hernández-Fuentes et al. 2015). The mid-elevation collection area was at 2800 m a.s.l., nearby La Parva ski resort (33º21'S, 70º19' W). At this elevation, the growing season usually lasts seven months (from October to April), the annual precipitation is 715 mm (falling predominantly as snow during winter) (Santibañez and Uribe 1990). Growing season mean temperature at this elevation is 10.7 °C with minimal and maximal temperatures of 3.7 and 21.4 °C, respectively (Hernández-Fuentes et al. 2015). The high-elevation collection area was at 3600 m a. s. l. (31°19’S, 70°15’W). This elevation has a 4–months growing season (from December to April), with an annual precipitation of 943 mm, again falling mainly as snow during winter with occasional hails or snow during summer (Santibañez and Uribe 1990). Thus, this site has no water limitations throughout the growing season (Sierra-Almeida and Cavieres 2010). Growing season mean temperature at this elevation is 8.2 °C with minimal and maximal temperatures of –1.0 and 20.1 °C (Hernández-Fuentes et al. 2015).

Experimental set up

We analyzed growth and storage during a period of darkness-induced C limitation and subsequent re-illumination, following a similar approach to Weber et al. (2018). The experiment was carried out with individuals collected in the field from the three elevations during the 2016–2017 growing season. We collected small seedlings (up to 4 ± 2 cm height, with 2–3 leaves) (i.e. after budburst), germinated during the same growing season of collection. Due to the remarkable elevational differences in the growing season, it was not possible to simultaneously collect plants at the same phenological stage from the different elevations, and to simultaneously expose them to the experimental conditions. Thus, plants from the different elevations were collected at different times and were then exposed to the same pre-experimental and experimental conditions, at different times. Plant collection was carried out in October, November and December at 1600, 2800, and 3600 m a.s.l., respectively. Then, the experiment extended from December to January for 1600 m plants, from January to February for 2800 m plants, and from February to March for 3600 m plants.

Plants were collected from the field by carefully digging the soil and then extracting the complete root system. Immediately after collection, plants were placed one by one on wet absorbent paper and kept under cool conditions. Plants were transported to a greenhouse (University of Concepción, Concepción, Chile) the day after collection. In the greenhouse, each plant was carefully transplanted into a 300 cc cell of speedling transplant trays (hereafter 'speedlings'; 88 cells per tray) containing a mixed substrate (commercial organic soil : sand, 1:1). The organic substrate (ANASAC®) had the following properties: pH (H2O): 5.25, mineral N: 43.4 mg kg–1, Olsen-P: 17.3 mg kg–1, organic matter: 22.4%, base saturation: 34.7 cmol+ kg–1) (Ávila-Valdés et al. 2019).The sand had neutral pH and was mostly composed by fine particles (apparent density 2.7 g cm-3). One month after transplant (i.e., November 2016, December 2016, and January 2017, for 1600, 2800 and 3600 m a.s.l. respectively), plants were placed in a walk-in growth chamber (PiTec S.A., Santiago, Chile) and maintained for 30 days under a constant air temperature of 17 °C, relative humidity average of 72%, soil moisture at field capacity, and 400 µmol m-2 s-1 of photosynthetic photon flux density (PPFD) with a day-length 15 h/9 h (day/night). After this period, 170 plants per elevation were randomly assigned to one of two light treatments, each replicated in 6 blocks (5 blocks with 28 plants and 1 block with 30 plants). In the first treatment (darkness–re-illumination), plants were exposed to 10 µmol m-2 s-1 of PPFD for 30 days (hereafter darkness) and then to 400 µmol m-2 s-1 of PPFD for another 30 days (hereafter re-illumination). The artificial darkness was created with black shade mesh (raschel, 80% shade) placed at ca. 20 cm over the speedlings. Black shade mesh covered both the top and sides of the speedlings, so light was homogeneously reduced around the plants. In the second treatment, plants were exposed continuously to 400 µmol m-2 s-1 of PPFD during daylight (hereafter control). The experiment was carried out in the same growth chamber were plants were acclimated. During the experiment, plants were kept in conditions of constant irrigation and temperature (15/5°C day/night).

One-to-two plants of each block and treatment were harvested every 15 days after the start of the experiment to assess growth and NSC concentrations. Additionally, a first harvest was carried out just before the start of the experiment. Thus, the first harvest (day 0) represented an initial condition, the 2nd and 3rd harvests corresponded to 15 and 30 days of darkness, respectively, while the 4th and 5th harvests corresponded to 15 and 30 days of re-illumination, respectively.

Growth measurements

Growth was measured as the whole-plant structural biomass increment after 15 and 30 days of darkness relative to the initial condition, and after 15 and 30 days of re-illumination. Seedlings were gently removed from pots and the roots were separated from the shoots and then washed with tap water (dead tissues attached to the plant were not excluded from the measurements). Shoots and roots were then stored separately in paper bags, dried at 70 °C in a forced-air oven (ZXFD-B5090, LABWIT Scientific Pty Ltd., Australia) for 4 days and then weighed in an analytical balance to obtain organ-specific biomasses and total plant biomass. The whole-plant NSC content (see below) was subtracted from the total plant biomass to obtain the structural biomass (Canham et al. 1999). The changes in biomass per individual (LRR_biomass) during the darkness period were estimated for each plant as the log10 of the ratio between the initial structural biomass (i.e., at day 0; bi) and the structural biomass after 30 days of darkness (bf):

$$LRR_{biomass} = \log 10\ \frac{bf}{bi},$$

Changes in biomass during the re-illumination period were analyzed similarly but in this case bi corresponded to the structural biomass of each plant after 30 days of darkness and bf corresponded to the structural biomass of each plant after 60 days from the beginning of the experiment (i.e. 30 days after re-illumination). Values > 0 indicate biomass increases; meanwhile values < 0 indicate biomass loss.

Carbohydrate analyses

Non-structural carbohydrate (NSC) concentrations were determined in the same plants harvested for biomass determination, for each tissue separately, as the sum of the quantitatively low molecular weight carbohydrates (i.e. glucose, fructose and sucrose, hereafter “total soluble sugars”: TSS) plus starch (as described in Hoch et al. 2002)). Low molecular weight sugars were extracted with distilled water at 100 °C for 60 min and analyzed photometrically at 340 nm on a 96-well multiplate reader after enzymatic conversion (invertase and phosphoglucose isomerase from Saccharomyces cerevisiae, Sigma Aldrich I4504 and P5381, respectively, St Louis, MO, USA) of sucrose and fructose to glucose. The concentration of glucose was determined photometrically after the enzymatic conversion of glucose to gluconate-6-phosphate (Glucose Assay Reagent, G3293 Sigma Aldrich). Following the degradation of starch to glucose, using a purified fungal amylase (‘amyloglucosidase’ from Aspergillus niger, Sigma Aldrich 10115) at 45 °C overnight, starch was determined in a separate analysis by the same procedure. The starch concentration was calculated as NSC minus the sum of low molecular weight carbohydrates and expressed as glucose equivalents. Concentrations are presented on a percent of dry matter basis (% d.m.).

To compare the NSC concentration between treatments, the mean NSC concentration for each plant was weighted, integrating the biomass and NSC concentration of each tissue, as described in Hoch et al. (2002), using the following formula:

$\sum_{org=1}^{n} \frac{conc_{org}\ x\ biom_{org}}{100}$,

where n is the number of organs, concorg is the organ-specific NSC concentration (% d. m.) and biomorg is the organ-specific fraction of the total biomass.

Changes in NSC concentration per individual (LRR_NSC) during the darkness period were estimated as the log10 of the ratio between the initial NSC concentration (i.e. at day 0; ci) of each plant and the NSC concentration after 30 days of darkness (cf) of each plant:

$$LRR\_NSC = \log_{10}(NSCcf/NSCci),$$

Changes in NSC concentration during the re-illumination period were analysed similarly but in this case ci corresponded to NSC concentrations of each plant after 30 days of darkness and cf corresponded to NSC concentrations of each plant after 60 days from the beginning of the experiment (i.e. 30 days after re-illumination) of each plant. Values > 0 indicate increase in NSC concentration, while values < 0 indicate decrease in NSC concentration.

Separately, fructans concentration was calculated using a method for reducing-sugars with a kit Megazyme Fructans HK Assay (AOAC Method 999.03 and AACC Method 32.32; Megazyme International Ireland Ltd., Wicklow, Ireland), following the procedure described by McCleary et al. (2000). Fructans were extracted by digesting 100 milligrams of sample in 40 ml of distilled water at 80 °C during 15 min. Two aliquots of the extract were treated with purified fructanase (fructanase; bottle 2 in Megazyme Fructans assay kit A98YV29) to hydrolyze fructans to fructose and another aliquot was treated with acetate buffer at 100 mM, pH: 4.5 (sample blank). Sugars concentration was measured at 410 nm with the para-hydroxybenzoic acid hydrazide (PAHBAH) method for reducing sugars.

To determine the fructans concentration, the following formula was used, as indicated in the kit: $\Delta A \times F \times \frac{V}{W} \times 2.48$; where, ΔA is absorbance mean between extracts treated with fructanase minus the absorbance of the blank, F is fructose factor to convert absorbance values to µg = (54.5 µg D-fructose)/(absorbance for 54.5 µg D-fructose), V is extraction volume, W is sample weight, and 2.48 corresponds to correction for all solutions during procedure. The whole-plant mean fructans concentration was estimated for each plant using the same procedure to estimate whole-plant NSC concentration.

Statistical analyses

To assess the effects of elevation, light condition (darkness or re-illumination versus control), date and the interactions among these factors on LRR_biomass and LRR_NSC, we used generalized linear mixed-effects models (GLMM), where gamma function better represented the data distribution. We utilized the function glmer in the R package nmle (Bates et al. 2014). In the analyses, the blocks were considered as a random factor. To analyse the allocation per individual to NSC and biomass after 30 days in darkness or after 30 days of re-illumination (relative to initial values and relative to the end of darkness, respectively), we used a linear mixed-effect model (LMM) with repeated measures. In these analyses, elevation was a fixed factor, LRRs after 30 days of darkness or after 30 of re-illumination the within-subject factor, and blocks were the random factor. The concentrations of compounds (starch, fructans, and total low molecular weight sugars) were analyzed using LMM, where blocks were the random factor and elevation, date, light treatment, and the interactions among them, were the fixed factors. LMM were performed with the function lmer in the R package nmle (Bates et al. 2014). Differences among treatments were tested using packages 'emmeans' and 'lsmean' (Lenth 2016). Tukey honest significant difference (HSD) was used to evaluate differences between pairs of means (alpha < 0.05). LMM and GLM were run in R (R 3.6.3).

Results

Growth responses

Control plants from the three elevations significantly increased their biomass after 30 days from the beginning of the experiment, although this increase was higher for plants from 1600 m than for plants from the other elevations (HSD Tukey test $p < 0.001$) (Fig. 2.2). Overall, plants in darkness decreased their biomass (significant effect of "darkness" on LRR_biomass, Figs 2.1 and 2.2), although this decrease differed among elevations depending on duration of darkness (significant effect of "elevation" × "light treatment (darkness)" × "date" on LRR_biomass, Fig. 2.1). In plants from 1600 m, LRR_biomass did not decrease significantly after 15 days of darkness (HSD Tukey test $p = 0.291$) but did so after 30 days of darkness (HSD Tukey test $p = 0.010$), although this decrease was small (Fig. 2.2a). In contrast, plants from 2800 and 3600 m decreased their biomass significantly both after 15 days (HSD Tukey test p =

0.032 and $p = 0.011$, respectively) and 30 days of darkness (HSD Tukey test $p < 0.001$ for both), reaching lower values than in plants from 1600 m (Fig. 2.2b and c).

During the re-illumination phase, control plants from the three elevations continued significantly increasing their biomass (Fig. 2.2d–f and Table 2.1). This increase was significantly higher in plants from 1600 m and 2800 m than in plants from 3600 m (HSD Tukey test $p < 0.001$ for both comparisons). Plants subjected to re-illumination after darkness also increased their biomass (Fig. 2.2d–f), in fact, at a higher rate than control plants in all cases (significant interaction effect of “light treatment” × “date” × “elevation” on LRR_biomass, Table 2.1 for “re-illumination”). Plants from 1600 m increased their biomass continuously and significantly after 15 and 30 days of re-illumination (HSD Tukey test $p < 0.001$ for both dates), exhibiting a 1.8 times higher increase than control plants (Fig. 2.2d). In contrast, plants from 2800 m showed an abrupt increase in biomass after 15 days of re-illumination (HSD Tukey test $p < 0.001$) and then did not significantly change until the end of the experiment (HSD Tukey test $p = 0.858$, Fig. 2.2e). The increase in re-illuminated plants from 2800 m was 2.5 times higher than in control plants (Fig. 2.2e). In plants from 3600 m, biomass after 30 days of re-illumination was smaller compared to plants from 1600 and 2800 m (Table A 2.1), but it was 3.9 times higher than the control plants (Fig. 2.2f).

Carbohydrate concentration responses

While control plants increased their NSC concentrations (relative to the initial values), plants under darkness significantly reduced their relative NSC concentrations (significant effect of “darkness” on LRR_NSC, Table 2.2 and Fig. 2.3a–c). Nonetheless, responses to darkness depended on the elevation (significant interaction effect of “elevation” × “date” × “light treatment” on LRR_NSC, Table 6.2). Control plants from 1600 and 2800 m presented a significantly lower LRR_NSC than plants from 3600 m after 30 days, while for the same date darkness caused a significantly lower decrease of LRR_NSC in plants from 3600 than in plants from 1600 m and 2800 m (HSD Tukey test $p < 0.001$) (Fig. 2.3a-c)

During the re-illumination phase, both control and re-illuminated plants increased their LRR_NSC. However, the magnitude of the increases depended on the elevation and on duration of re-illumination (significant interaction effect of “light treatment re-illumination” × “date” × “elevation” on LRR_NSC, (significant interaction effect of “light treatment re-illumination” × “date” × “elevation” on LRR_NSC, Table 2.2, Fig. 2.3d–f). After 15 days, both control and re-illuminated plants from 1600 m showed no increase in LRR_NSC; significant increases in re-illuminated plants were observed during the second period of the re-illumination phase (HSD Tukey test $p < 0.001$), when re-illuminated plants showed 6.4 times higher increase than control plants (Fig. 2.3d). In contrast, for both control and re-illuminated plants from 2800 m and 300 m, LRR_NSC increased progressively during this experimental phase (HSD Tukey test p < 0.001 for 2800 and 3600) (Fig. 2.3e and f) and the increase in re-illuminated plants relative to controls was lower than for 1600 m plants; for plants from 2800 m the increase was 2.7 times higher than in control plants (Fig. 2.3e), while in plants from 3600 m it was 3.7 times higher than in control plants (Fig. 2.3f).

Growth versus Storage

After 30 days of darkness, plant biomass was significantly less reduced than NSC concentrations (Figs. 2.4a–c). However, these responses depended on the elevation provenance (significant interaction effect of “elevation” × “LRR after 30 days of darkness” on LRR_biomass and LRR_NSC after 30 days of darkness, Table 2.3). The decrease in LRR_NSC was 12.5-fold greater than the decrease in LRR_biomass resulting from leaf shedding (HSD Tukey test p < 0.001, Fig. 2.4a) for plants from 1600 m, but only 2.8-fold and 1.7-fold greater for plants from 2800 m and 3600, respectively (HSD Tukey test p < 0.001 and p = 0.012, respectively, Figs. 2.4b and 2.4c).

After 30 days of re-illumination, plants from the different elevations showed recovery in both biomass and NSC concentration with respect to 30 days of darkness. For all elevations, increases in NSC concentration were higher than increases in biomass, although their magnitudes depended on the elevation provenance (significant interaction effect of “elevation” × “LRR after 30 days of re-illumination” on LRR_biomass and LRR_NSC after 30 days of re-illumination with respect to 30 days of darkness, Table 2.3, Figs. 2.4d–f). In plants from 1600 m, the increase in LRR_NSC was 1.5-fold higher than

LRR_biomass, (HSD Tukey test p = 0.003, Fig. 2.4d). By contrast, the increase in LRR_NSC was 2.1- and 1.9-fold significantly higher than the increase in LRR_biomass in plants from 2800 m and 3600 m, respectively (HSD Tukey test p < 0.001 for both, Figs. 2.4e and 2.4f).

Starch, fructans and total soluble sugars

Elevation, light conditions, time and all their interactions significantly affected the concentration of starch, fructans and TSS (Table 2.4). Although control plants from all elevations showed an increase in starch, fructans and TSS concentrations during the experiment, starch was the main C storage form in 1600 m plants. Further, in plants from this elevation, darkness caused decreases in all fractions, while TSS had a higher recovery than starch and fructans after re-illumination (Fig. 2.5d). In contrast, starch was the main compound that increased during the experiment in control plants from 2800 m, while fructans concentration was lower and remained constant over the whole experimental period (Fig. 2.5b). During the period of darkness and subsequent re-illumination, the concentration of all compounds varied similarly in plants from 2800 m (Fig. 2.5e). Finally, all fractions had similar concentrations in plants from 3600 m, although starch represented a lower fraction by the end of the experimental period (Fig. 2.5c). Also, the response to re-illumination of plants from 3600 m was characterized by a greater recovery of fructans than starch and TSS, both after 15 and 30 days of re-illumination (Fig. 2.5f).

Discussion

We found that the dynamics of biomass and carbohydrate storage in response to darkness and re-illumination differed between elevational provenances in a widely-distributed alpine plant species. By evaluating the storage and biomass in *Phacelia secunda* plants from three elevations of the Mediterranean Andes of Chile, we found that under C limitation (darkness) plants from all elevations reduced proportionally more their NSC concentrations than their biomass. However, the magnitude of such reductions differed among elevation provenance: the reduction in NSC concentration was 12.5-fold greater than the reduction in biomass in low-elevation plants, but only 2.8 and 1.7-fold greater in mid- and high-elevation plants, respectively. These results are consistent with a higher importance of allocation to storage relative to biomass in mid- and high-elevation plants compared to low elevation plants. Conversely, biomass formation appeared relatively more important in low than mid- and high-elevation plants. Similar elevational differences in C allocation priorities can be also inferred from biomass and NSC changes during re-illumination, when increases in NSC concentration relative to increases in biomass were higher in high- than in low-elevation plants (1.9 and 1.5, respectively). In addition, while low-elevation plants started to increase the biomass soon after the beginning of re-illumination, they increased their NSC concentration only during the second half of the re-illumination period. By contrast, high-elevations plants started to recover both C sinks immediately after re-illumination. Thus, our study suggests that C allocation to growth and storage in alpine plants is not only a result of short-term responses to environmental cues but may also reflect population differences (e.g. ecotypic), as it has been previously proposed (Smith et al. 2003; Wiley and Helliker 2012).

Although we inferred C allocation priorities based on two light treatments (darkness or re-illumination), responses during re-illumination are likely more conclusive. Darkness provoked a negative C balance and plants had to remobilize their C reserves for survival during this period. Instead, responses of C-limited plants during re-illumination indicate the fate of C right after a positive C gain was resumed (Weber et al. 2018). However, NSC changes during darkness may also be informative on allocation priorities, as the rate of remobilization of C reserves during C limitation is potentially dependent on the metabolic demands. For example, interspecific differences in allocation to growth and to storage are exhibited amongst species of contrasting light requirements even under C limitation (Kitajima 1994). Similarly, decreases in structural biomass during darkness should be associated with a reduction in the C costs of tissue maintenance (Piper and Fajardo 2016; Weber et al. 2018). Thus, the decrease in structural biomass observed in high-elevation plants (mostly driven by leaf shedding according to our personal observations) probably meant less biomass to maintain and hence a saving of C reserves.

Contrary to what we expected, our results pointed to a lesser importance of allocation to storage (relative to biomass) in low elevation plants compared to high-elevation plants. This result suggests that plants from 1600 m prioritized biomass formation relative to reserve formation, especially when compared to plants from higher elevations. At this elevation, temperature and soil moisture conditions are favorable for both photosynthesis and growth during early spring and these favorable conditions last for a couple of months before the onset of drought (Peñaloza et al. 2001) when, as in other Mediterranean systems (Körner 2003b), growth ends by early summer as a consequence of severe drought. Indeed, stomatal conductance of *P. secunda* at this time is 84% lower than at the highest elevation (Hernández-Fuentes et al. 2015). Thus, the apparent prioritization to growth over storage in low-elevation plants could relate to the phenology of these plants, characterized by an early occurrence of the growing season and a very short duration of snow cover compared to higher elevations. A shorter period of the snow cover probably determines a lower demand of C reserves during winter. Thus, it would not be necessary to start winter with too high levels of C reserves. On the other hand, the risk of C starvation during summer, which we had hypothesized to favor a prioritization of C allocation to storage, might be not as severe. It has been found that under the frequent summer drought at 1600 m *P. secunda* is still able to assimilate some C (Hernández-Fuentes et al. 2015), which is probably sufficient to meet its metabolic demands and in turn to minimize the risk of C starvation. On the other hand, the vegetation cover and species richness are higher at lower than at high-elevations in central Chile (Cavieres et al. 2000), which might imply a higher competition for resources and space. In this case, fast growth would confer more benefits than storage.

The greater importance of allocation to storage (relative to biomass) in mid- and high- elevation plants compared to low-elevation ones after severe C starvation, likely reflects a strategy to withstand harsh environmental conditions that could cause C limitation and C starvation. For example, the long duration of the snow cover period characterizing higher elevations forces plants to survive at the expense of their C reserves, and often to start budbreak and growth under snow. For instance, Körner et al. (2019) recently reported that the alpine plant *Soldanella pusilla* resumes growth during the winter under 2–3 m of snow, thanks to the large amount of NSC that this species accumulated during the summer (at the beginning of winter NSC concentration accounted for ca 50% of the dry matter of leaves and roots). Indeed, that study showed that about one-third of the reserves of leaves and a smaller fraction (a drop by 9%) of below-ground tissue were consumed by metabolism and growth at mid-winter, demonstrating the importance of C storage for winter survival and for a fast growth and reproduction as soon as snow melts. In addition, freezing temperatures during the growing season can cause significant tissue damage (a form of defoliation) and plants usually replace this tissue lost within the growing season. Further, at high elevations, plants are also more subjected to environmental disturbances such as sudden changes in cloud cover and summer snow (see climate data Sierra-Almeida and Cavieres (2010)), which may strongly limit the light availability for photosynthesis.

At first glance, the biomass increases after re-illumination exhibited by C limited plants (i.e. after 30 days of darkness) relative to controls, that were higher in high-elevation than in mid- and low- elevations plants (Fig. 2.2 and Table A2.5), seems opposite to the idea of a lower importance of C allocation to biomass in these plants than in their low-elevation counterparts. The higher biomass increase of high-elevation plants could be related to the natural seasonal cycles in the field. High-elevation plants experience longer periods of a deep snow cover (i.e. darkness) during winter months than low-elevation plants. Indeed, this plant species loses its foliage under the snow and fully regrows during spring (C. Reyes-Bahamonde and L.A. Cavieres, personal observations). Thus, although growth likely starts before snow melting in high elevation plants (see above), it is possible that light regulates developmental transitions (e.g. triggers fast re-growth) in high-elevation populations(e.g. Kami et al. 2010). By contrast, light is probably not a strong signal for growth initiation in low-elevation plants, since the period of snow cover during the winter is much shorter (there are years with no snow cover, indeed), and the beginning of the growing season is not related to snow melting. Despite the greater biomass increase shown by high-elevation plants than by low-elevation plants relative to their corresponding controls, it is important to note that high-elevation plants showed a much higher increase in NSC concentration than in biomass with respect to control plants (i.e. 8.8 and 3.22 times for NSC concentration and biomass in plants subjected to darkness–re-illumination, respectively, versus 1.79 and 1.33 times for NSC concentration and biomass in control plants, respectively, Table A2.5, Fig. 2.2 and Fig. 2.3). Therefore, when results of C allocation in high-elevation plants during re-illumination are interpreted relative to control plants, they still suggest a shift in C allocation, showing higher importance of C allocation to storage over growth.

Our results also suggest ecotypic differences in the fractions of NSC. After re-illumination, TSS concentration recuperated more than starch and fructans in plants from 1600 m (Fig. 2.5d). This could be an intrinsic response of low-elevation plants, since they are naturally subjected to summer drought (Hernández-Fuentes et al. 2015). Some soluble sugars act as osmotic agents, helping to maintain the physical structure and (supra-) molecular components of cell membranes. The increase in concentration of soluble sugars with osmotic activity is an important adaptation of plants to drought stress (Handa et al. 1983; Körner 2015). Our results agree with those of García-Lino et al. (2017), who found that *Laretia acaulis*, an alpine cushion plant from central Chile, had high concentrations of low-weight molecular sugars at low and mid-elevations throughout the growing season, which could reflect a high C investment in osmoregulation and osmoprotection. Thus, although storage did not seem to be a priority of allocation in plants from the lowest and driest elevation, the preferential formation of sugars rather than of starch and fructans suggests that storage was highly regulated (i.e. C allocation for osmoregulation).

After a period of C limitation, plants from high-elevations tended to restore their C reserves, accumulating fructans and –to a less extent– starch (Fig. 2.5f). Fructans are recognized as one of the main forms of C storage in c. 12% of higher plants, even some families (i.e. Asteraceae) store fructans only (Hendry et al. 1993). Vegetative tissues of fructans-rich species have generally low concentrations of starch (Hendry 1987; Pollock 1986). Although the predominant role of fructans is to bridge temporal gaps between resource availability and demands, Chatterton et al. (1989) indicated that fructans accumulation is not an alternative to starch formation. They described fructans as an auxiliary form of carbohydrate storage that accumulates in the vacuole to allow photosynthesis to continue at cool temperatures when other storage pools are saturated. However, it has been observed that fructans can also regulate osmosis during bloom (Le Roy et al. 2007), and protect against freezing and drought stress through membrane stabilization (Hincha and Hagemann 2004). Since in plants from 3600 m fructans were significantly reduced by darkness but were soon recovered during re-illumination, our results suggest that in plants of this elevation fructans could play both storage and cryoprotective roles.

We expected that predictions iii) and iv) were be particularly evident in plants from the high- and low-elevations, but not for mid-elevation plants. In contrast, we found that after the darkness period, plants from mid-elevation responded similar to low-elevation plants showing a high decrease in NSC concentration, but decreased their biomass like high-elevation plants. However, the difference between decreases in NSC concentration and biomass were not similar to high- or low-elevation plants (Fig. 2.4b). This response could indicate that mid-elevation plants present high NSC utilization during a period of darkness, such as low-elevation plants, but a significant loss of biomass due to leaf shedding, such as high-elevation plants. Therefore, in plants from mid-elevation, a more balanced allocation to both storage and growth could reflect less stressful condition at this elevation. On the other hand, plants from mid-elevation showed higher NSC concentration recovery than biomass after re-illumination (Fig. 2.4e). Contrary to what we expected, this recovery was much greater than in the other two elevations, suggesting that among the three provenances mid-elevation plants prioritized storage relative to growth. A possible explanation would be that the photosynthesis rates of plants from 2800 m are higher and/or respiration rates are lower than that of plants from the other elevations. However, we have no data to support this explanation and thus, further research is needed to elucidate drivers of C allocation at the mid-elevation.

Our results are consistent with the view of C storage in alpine plants as a sink itself (i.e. carbon reserves formation, sensu Chapin III et al. 1990) and not only as the result of an accumulation driven by the higher sensitivity of growth than of photosynthesis to low temperatures (Boyer 1970; Muller et al. 2011). All plants, but more so those from mid- and high-elevations, increased their NSC concentrations after a period of C limitation and at the same time that they increased their structural biomass. As a potential explanation for the lower allocation to storage in low than mid- and high-elevation plants, we suggest that the occurrence of summer drought by the end of the growing season, associated with non-zero C gain (Hernández-Fuentes et al. 2015), promotes levels of C accumulation which are sufficient to avoid C starvation during summer drought and to meet C demands during winter. Our study therefore suggests that low temperature, but not drought, favours C allocation to storage over growth. Nonetheless, the preferential formation of sugars over starch exhibited by low-elevation plants demonstrates that storage is highly regulated in drought-adapted plants.

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Tables

Table 2.1 Results from generalized linear mixed-effects models (GLMM) testing the effects of elevation (1600, 2800 and 3600 m a.s.l), light condition (darkness or re-illumination versus control), date (0, 15, 30, 45, 60 days) and the interactions among these factors on the LRR_biomass, during darkness and re-illumination period in the herbaceous alpine plant *Phacelia secunda* from the Mediterranean Andes of central Chile (Fig. 2.2).

Factor	d.f.	Chisq	Pr(<Chisq)
LRR_biomass darkness			
Date	2	5.37	0.068
Light treatment (darkness)	1	156.17	<.001
Elevation	2	40.95	<.001
Light treatment (darkness) * Date	2	102.43	<.001
Date * Elevation	4	25.94	<.001
Light treatment (darkness) * Elevation	2	22.98	<.001
Light treatment (darkness) * Date * Elevation	4	13.95	0.007
LRR_biomass re-illumination			
Date	2	1717.87	<.001
Light treatment (darkness)	1	683.66	<.001
Elevation	2	254.53	<.001
Light treatment (darkness) * Date	2	198.71	<.001
Date * Elevation	4	71.41	<.001
Light treatment (darkness) * Elevation	2	84.415	<.001
Light treatment (darkness) * Date * Elevation	4	18.59	<.001

d.f. stands for degrees of freedom.

Table 2.2 Results from generalized linear mixed-effects models (GLMM) testing the effects of elevation (1600, 2800 and 3600 m a.s.l), light condition (darkness or re-illumination versus control), date (0, 15, 30, 45, 60 days) and the interactions among these factors on the LRR_NSC, during darkness and re-illumination period in the herbaceous alpine plant *Phacelia secunda* from the Mediterranean Andes of central Chile (Fig. 2.3).

Factor	d.f.	Chisq	Pr (>Chisq)
LRR_NSC darkness			
Date	2	71.00	<.001
Light treatment (darkness)	1	347.59	<.001
Elevation	2	24.47	<.001
Light treatment (darkness) * Date	2	444.37	<.001
Date * Elevation	4	31.81	<.001
Light treatment (darkness) * Elevation	2	34.09	<.001
Light treatment (darkness) * Date * Elevatior	4	57.89	<.001
LRR_NSC re-illumination			
Date	2	1052.09	<.001
Light treatment (darkness)	1	537.49	<.001
Elevation	2	555.96	<.001
Light treatment (darkness) * Date	2	181.35	<.001
Date * Elevation	4	245.31	<.001
Light treatment (darkness) * Elevation	2	88.13	<.001
Light treatment (darkness) * Date * Elevatior	4	183.69	<.001

d.f. stands for degrees of freedom.

Table 2.3 Results from a linear mixed-effects models (LMM) with repeated measures testing changes magnitudes in NSC concentrations and biomass after 30 days in darkness and after 30 days of re-illumination (relative to initial values and relative to end of darkness, respectively), between elevations (1600, 2800 and 3600 m a.s.l) in the herbaceous alpine plant *Phacelia secunda* from Andes (Fig. 2.4)

Factor	d.f.$_{num}$	F value	p-value
LRR_biomass and LRR_NSC after 30 days of dar			
Elevation	2	14.417	<.001
LRR after 30 days of darkness	1	232.468	<.001
Elevation: LRR after 30 days of darkness	2	19.067	<.001
LRR_biomass and LRR_NSC after 30 days of re-			
Elevation	2	73.493	<.001
LRR after 30 days of re-illumination	1	178.450	<.001
Elevation: LRR after 30 days of re-illumination	2	19.925	<.001

d.f.num stands for degrees of freedom of the numerator, degrees of freedom for denominator (d.f.den) = 30.

Table 2.4 Results from a linear mixed-effects models (LMM) testing the effects of elevation (1600, 2800 and 3600 m a.s.l), light condition (darkness or re-illumination versus control), date (0, 15, 30, 45, 60 days) and the interactions among these factors on the concentrations of compounds (starch, fructans, and total low molecular weight sugars), during darkness and re-illumination period, in the herbaceous alpine plant *Phacelia secunda* from the Mediterranean Andes of central Chile (Fig. 2.5)

Factor	d.f.$_{num}$	F-value	p-value
Starch concentration (%DM)			
Date	4	569.74	<.0001
Light condition	1	4255.92	<.0001
Elevation	2	1003.36	<.0001
Light treatment * Date	4	439.14	<.0001
Date * Elevation	8	96.39	<.0001
Light treatment * Elevation	2	595.69	<.0001
Light treatment * Date * Elevation	8	11.95	<.0001
TSS concentration (%DM)			
Date	4	116.06	<.0001
Light condition	1	908.19	<.0001
Elevation	2	44.87	<.0001
Light treatment * Date	4	69.25	<.0001
Date * Elevation	8	16.61	<.0001
Light treatment * Elevation	2	22.41	<.0001
Light treatment * Date * Elevation	8	6.34	<.0001
Fructans concentration (%DM)			
Date	4	515.42	<.0001
Light condition	1	3091.82	<.0001
Elevation	2	190.40	<.0001
Light treatment * Date	4	232.87	<.0001
Date * Elevation	8	66.77	<.0001
Light treatment * Elevation	2	191.90	<.0001
Light treatment * Date * Elevation	8	49.67	<.0001

d.f.num stands for degrees of freedom of the numerator, degrees of freedom for denominator (d.f.den) = 145.

Figures

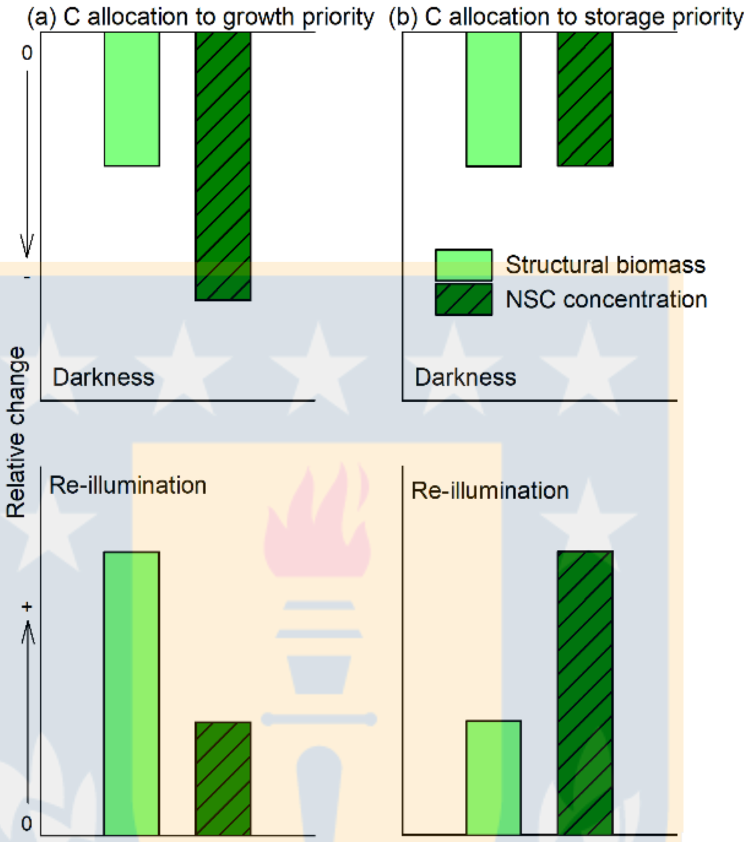

Figure 2.1 Alternative responses of non-structural carbohydrate (NSC) concentration and growth to darkness and re-illumination. (a) Growth is a priority of C allocation relative to storage, thus NSC concentrations decrease significantly in darkness, and increases in biomass caused by re-illumination should start earlier, and probably be higher than increases in NSC concentrations. (b) C allocation to storage is a priority relative to allocation to growth. Decreases in NSC concentrations caused by darkness should be less pronounced than in a) and increases in NSC concentrations caused by re-illumination should be higher than increases in biomass. In darkness, decreases in structural biomass caused by dead tissue (e.g. leaf shedding) are expected to be similar.

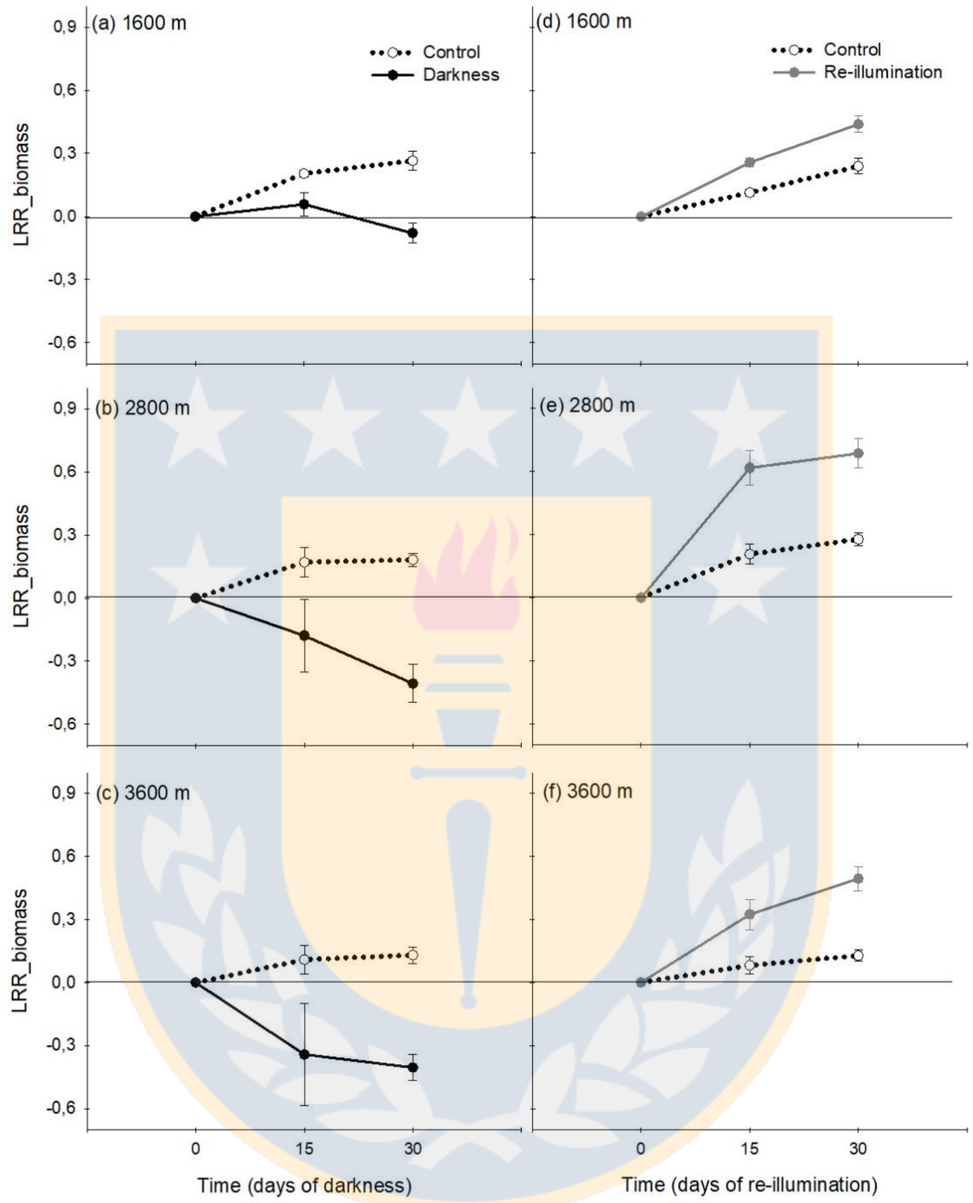

Figure 2.2 Log10 of the ratio between structural biomass after 30 days of darkness and initial structural biomass in the herbaceous alpine plant *Phacelia secunda* from three elevations of the Mediterranean Andes of Central Chile: 1600 m (a), 2800 m (b) and 3600 m (c) (left panel). Log10 of the ratio between structural biomass after 30 days of re-illumination and structural biomass after 30 days of darkness in *Phacelia secunda* from three elevations of the Mediterranean Andes of central Chile: 1600 m (d), 2800 m (e) and 3600 m (f) (right panel). Values > 0 and < 0 indicate increase and decrease in biomass, respectively. Error bars denote ± 2SE of the mean (n=6).

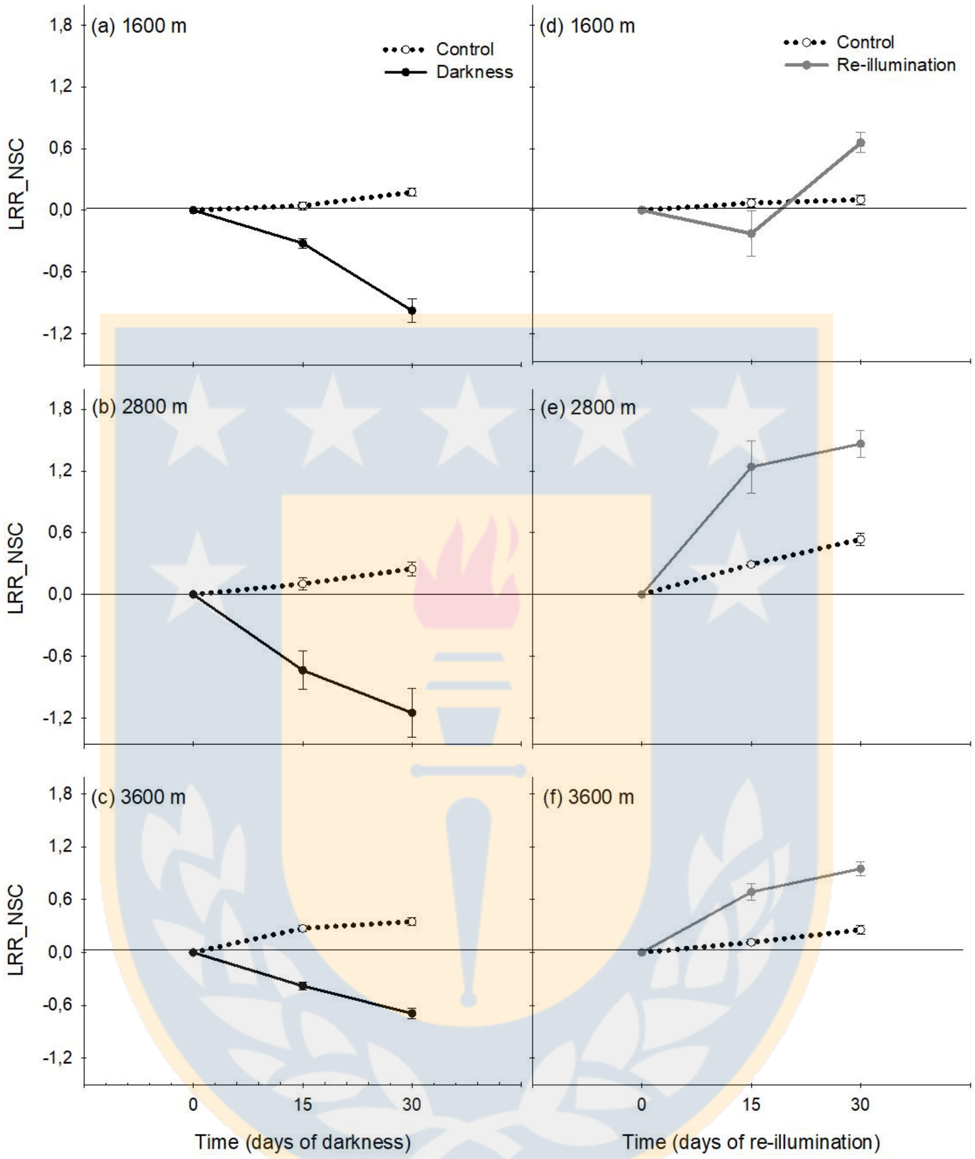

Figure 2.3 Log10 of the ratio between non-structural carbohydrate (NSC) concentration after 30 days of darkness and initial NSC concentration in the herbaceous alpine plant *Phacelia secunda* from three elevations of the Mediterranean Andes of central Chile: 1600 m (a), 2800 m (b) and 3600 m (c) (left panels). Log10 of the ratio between NSC concentration after 30 days of re-illumination and NSC concentration after 30 days of darkness in *Phacelia secunda* from three elevations of the Mediterranean Andes of Central Chile: 1600 m (d), 2800 m (e) and 3600 m (f) (right panels). Values > 0 and < 0 indicate increase and decrease in NSC concentration, respectively. Error bars denote ± 2SE of the mean (n=6).

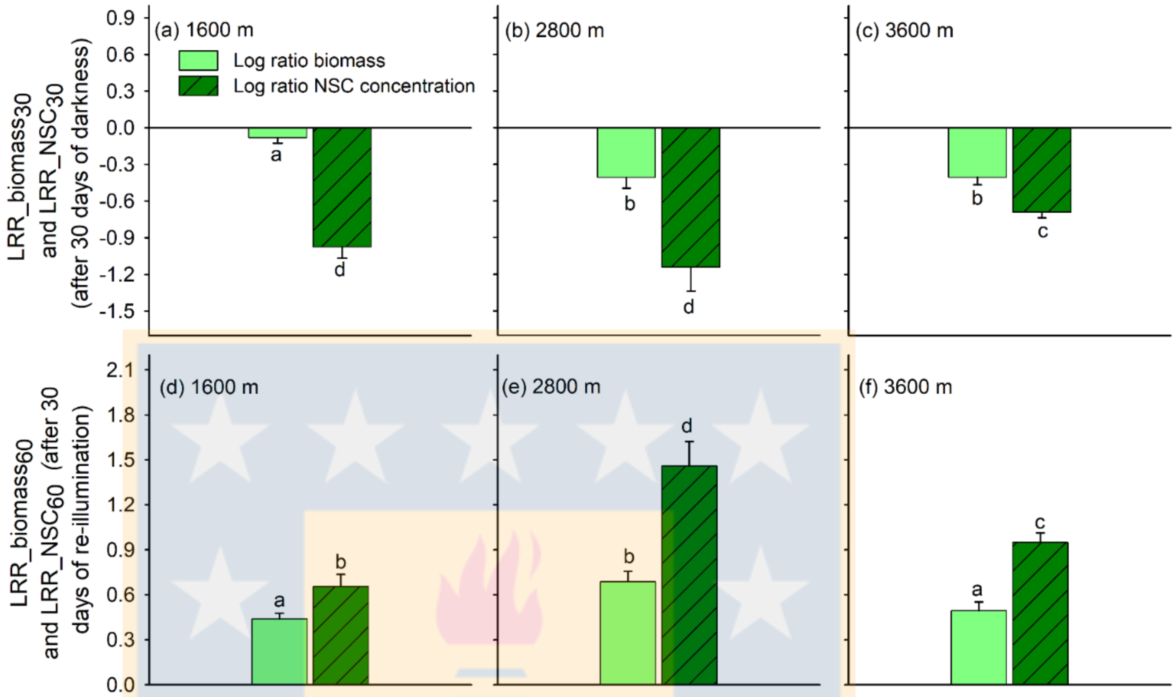

Figure 2.4 LRR_biomass30 and LRR_NSC30 (after 30 days of darkness, relative to the initial day) in the herbaceous alpine plant *Phacelia secunda* from three elevations of the Mediterranean Andes of Central Chile: 1600 m (a), 2800 m (b) and 3600 m (c) (top panels). LRR_biomass60 and LRR_NSC60 (after 30 days of illumination relative to 30 days of darkness) in *Phacelia secunda* plants from three elevations of the Mediterranean Andes of central Chile: 1600 m (d), 2800 m (e) and 3600 m (f) (bottom panels). Different letters denote statistically significant ($p < 0.05$). Error bars denote ± 2SE of the mean (n=6).

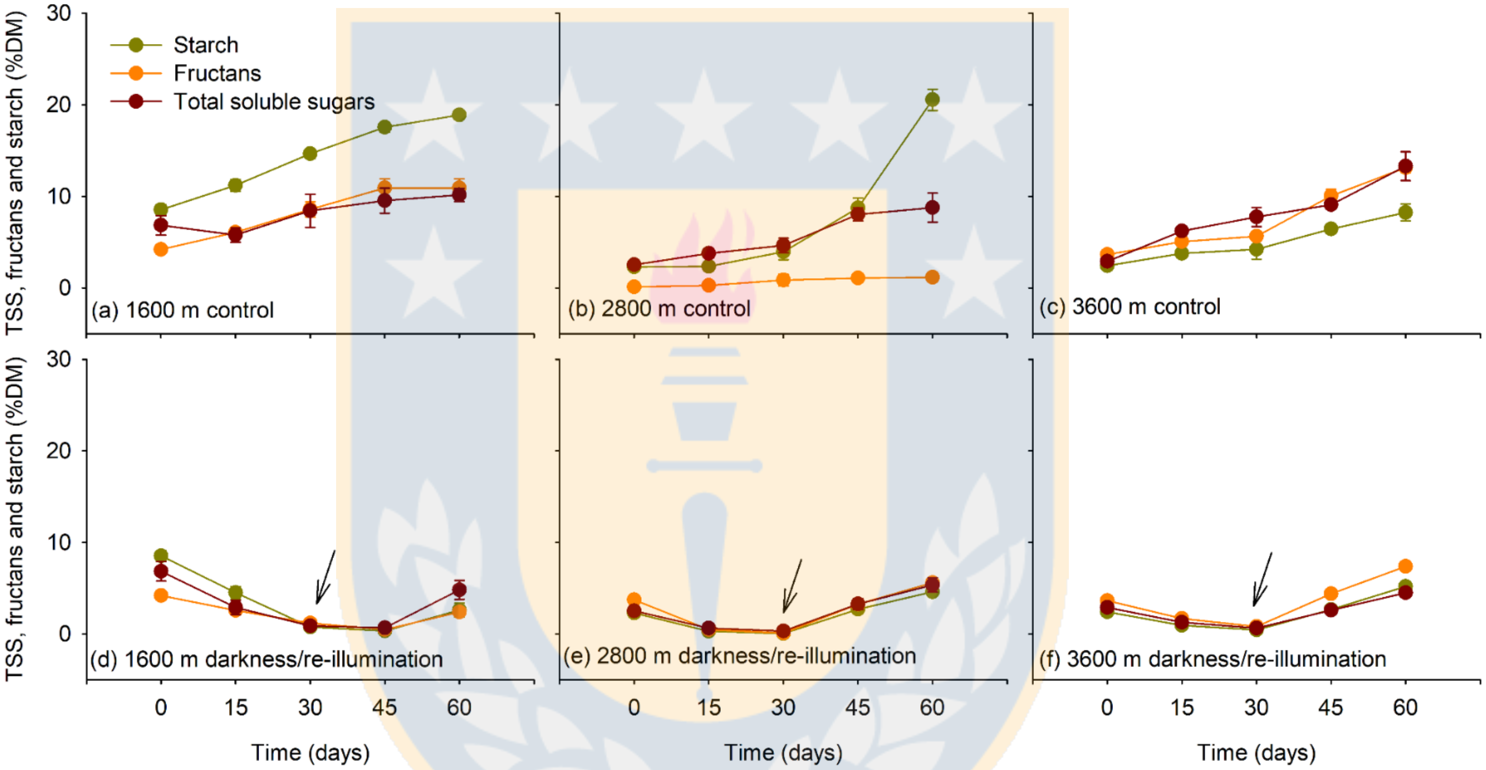

Figure 2.5 Concentrations of starch, fructans and total soluble sugars (TSS) in the herbaceous alpine plant *Phacelia secunda* from 1600 m, 2800 m and 3600 m of the Mediterranean Andes of Central Chile. Figures a, b and c correspond to control light conditions, while d, e and f correspond to darkness and subsequent re-illumination. Arrow denotes the beginning of re-illumination in the darkness condition. Error bars denote ± 2SE of the mean (n=6).

Supplementary Material

Table 2.5 Structural biomass and non-structural carbohydrates (NSC) concentration at different experimental dates in plants of *Phacelia secunda* from three elevations of central Chile, subjected to two light treatments in nursery conditions at University of Concepción, Chile: Control (400 µmol m^{-2} s^{-1} PPFD) versus Darkness–re-illumination (30 days of darkness followed by 30 days of 400 µmol m^{-2} s^{-1} PPFD). Arrows denote the beginning of re-illumination in the darkness condition.

Biomass (g)

	1600 m		**2800 m**		**3600 m**	
	Control	**Darkness–re-illumination**	**Control**	**Darkness–re-illumination**	**Control**	**Darkness–re-illumination**
0 days	0.26 ± 0.02	0.26 ± 0.02	0.19 ± 0.01	0.19 ± 0.01	0.22 ± 0.02	0.22 ± 0.02
15 days	0.42 ± 0.02	0.31 ± 0.04	0.29 ± 0.04	0.14 ± 0.04	0.28 ± 0.02	0.12 ± 0.05
30 days ←	0.48 ± 0.03	0.22 ± 0.02	0.29 ± 0.01	0.08 ± 0.01	0.30 ± 0.01	0.09 ± 0.01
45 days	0.63 ± 0.02	0.40 ± 0.04	0.47 ± 0.04	0.32 ± 0.06	0.36 ± 0.03	0.19 ± 0.03
60 days	0.84 ± 0.05	0.60 ± 0.03	0.55 ± 0.02	0.37 ± 0.04	0.40 ± 0.02	0.28 ± 0.05

NSC concentration (% dry mass)

	1600 m		**2800 m**		**3600 m**	
	Control	**Darkness–re-illumination**	**Control**	**Darkness–re-illumination**	**Control**	**Darkness–re-illumination**
0 days	15.38 ± 0.84	15.38 ± 0.84	4.82 ± 0.43	4.82 ± 0.43	5.32 ± 0.19	5.32 ± 0.19
15 days	16.98 ± 0.61	7.41 ± 0.86	6.13 ± 0.56	0.94 ± 0.28	9.99 ± 0.32	2.23 ± 0.15
30 days ←	23.08 ± 1.73	1.66 ± 0.31	8.58 ± 0.79	0.38 ± 0.14	11.99 ± 0.90	1.10 ± 0.16
45 days	27.08 ± 1.29	1.03 ± 0.30	16.75 ± 1.02	5.99 ± 0.73	15.53 ± 0.44	5.27 ± 0.22
60 days	29.03 ± 0.80	7.43 ± 0.99	29.31 ± 0.96	9.96 ± 0.77	21.53 ± 0.79	9.69 ± 0.47

CAPÍTULO III CARBON RESERVES INCREASE DROUGHT SURVIVAL IN AN ALPINE SPECIES UNDER MEDITERRANEAN CLIMATE

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Abstract

During periods of reduced photosynthesis due to drought, shade or defoliation, non-structural carbohydrates (NSC) can be used to maintain basic metabolic functions and to fuel compensatory growth. Thus, greater NSC concentration could be part of a preventative strategy against potential C starvation driven by long periods of reduced photosynthesis due to drought. It has been observed that high C storage could increase survival under water stress conditions. Alpine habitats in Mediterranean-type climate zones experience summer drought restricting the photosynthesis and growth, especially at lower elevations. Here we assessed whether NSCs confer survival advantages under drought in low elevation alpine plants of a Mediterranean region. If NSC are stored as a preventative strategy against potential C starvation (driven by drought and warmer conditions) then, plants from low elevation should remobilize more NSC storage than high elevation plants in response to drought, or to account for higher NSC levels. We exposed *P. secunda* plants from three elevations to factorial experiment with two growth temperature conditions (15/7 and 5/2°C) and two drought levels (mild drought and severe drought) for a month. Our results showed that plants from low and mid elevation presented higher survival to drought than plants from the highest elevation. Severe drought caused a reduction in NSC concentrations of high elevation plants, regardless of temperature. By contrast, in low elevation plants, severe drought caused decreased NSC concentrations only under 5/2 °C, while under 15/7 °C NSC concentrations and particularly totao soluble

sugars (TSS) were actually higher regarding control plants. As a result, low- and mid-elevation plants had higher NSC and TSS concentrations relative to high elevation plants, but not greater NSC remobilization, under the combination of severe drought and warming. Low elevation plants were more drought resistant than upper elevation plants, consistent with local adaptation to drought. Mid elevation plants had intermediate drought resistance. The high TSS concentration could be an intrinsic response of low-elevation plants, since they are naturally subjected to summer drought, because the increase in concentration of soluble sugars with osmotic activity is an important adaptation of plants to drought stress.

Introduction

Non-structural carbohydrates (NSC) confer tolerance to stress (Myers and Kitajima 2007; O'Brien et al. 2014; Piper and Paula 2020). During periods of reduced photosynthesis due to low soil water availability, shade, or defoliation, NSC can be used to maintain basic metabolic functions and to fuel compensatory growth (Chapin et al. 1990; Dietze et al. 2014). In most plants NSC are mainly formed by low molecular weight sugars and starch. While sugars can be mobilized quickly and used for short-term metabolism or osmotic functions, starch has no other known function than storage and can be stored for longer periods and then subequentently used during severe stress (Carbone et al. 2013; Chapin et al. 1990; Dietze et al. 2014). Likewise starch, fructans have been also considered to serve mainly as storage carbohydrates (Archbold 1940; Smith 1972). In many species they are the major carbohydrate reserves, particularly among the Compositae and Liliaceae families. In other families, they are the main carbohydrate remobilized during early vegetative growth (Gramineae and Boraginaceae). Fructans are often found in large concentrations in underground storage organs of geophytes such as *Galanthus nivalis* (Amaryllidaceae) and *Allium* species (Alliaceae), where they can constitute more than 40% of the dry mass (Orthen and Wehrmeyer 2004; Ranwala and Miller 2008).

High NSC concentrations have been widely observed in plants growing in habitats characterized by water shortages (Hoch and Körner 2003; Sala and Hoch 2009; Zhang et al. 2015), and they have been interpreted to reflect greater restrictions on growth (sink limitation) than on photosynthesis (source limitation) (Boyer 1970; Domisch et al. 2001; Kontunen-Soppela et al. 2002; Muller et al. 2011). Indeed, growth is usually much more sensitive than photosynthesis to drought. The levels of water stress that limit growth can be significantly smaller than those causing complete stomatal closure, and photosynthetic rates can be only slightly reduced when growth ceases (Muller et al. 2011). Thus, high annual NSC concentrations could mirror greater restrictions on tissue formation (C-sink limitation) than on photosynthesis (C-source limitation), driven by water shortage conditions. An alternative explanation for the high annual NSC concentrations is a priority of C allocation to storage, potentially occurring at the expense of growth (Wiley and Helliker 2012). This hypothesis proposes that greater NSC concentration could be part of a preventative strategy against potential C starvation driven by long periods of reduced photosynthesis due to drought (Wiley and Helliker 2012).

Therefore, the amount of carbon stored and the way that plants manage them may have adaptive value under specific environmental scenarios. It has been observed that high C storage could prolong survival under conditions water stress (Canham et al. 1999; O'Brien et al. 2014; Poorter and Kitajima 2007). Although a tradeoff between growth and storage has been recently demonstrated (Blumstein et al. 2022), and allocation to C storage may take place before C allocation to growth after re-illumination of C-starved plants (Reyes-Bahamonde et al. 2021; Weber et al. 2018), whether the prioritization of C allocation to storage confers drought survival remains unclear.

Phacelia secunda is an alpine plant species inhabiting mountains with Mediterranean-type climate. As in any other elevational gradient low temperature limitations increase with the elevation determining shorter growing seasons. Due to the Mediterranean climate, there is also summer drought restricting the photosynthesis and growth, especially at lower elevations (Hernández-Fuentes et al. 2015; Reyes-Bahamonde et al. 2022). At this elevation, drought is usually accompanied by warming. High temperatures, can modulate plant water use through its effects on vapor pressure deficit (VPD), so higher temperatures may exacerbate the effects of drought (McDowell et al. 2008; Will et al. 2013; Williams et al. 2013). Thus at low elevation, drought and warming would intensify the C storage dependence. Previous studies have found that *P. secunda* in both low and high elevations has high annual NSC concentrations, and that C-starved plants of both elevations quickly increase NSC concentration in response to re-illumination, suggesting that storage is a priority sink (Reyes-Bahamonde et al. 2021). We here ask whether NSCs confer survival advantages under drought. We expect that if NSC are stored as a preventative strategy against potential C starvation driven by dry and warm conditions, plants from low elevation should remobilize more NSC storage and show higher survival than high elevation plants, when both are compared in response to such conditions. To test these expectations, we exposed seedlings of *P. secunda* from three elevations, to a bifactorial experiment combining three levels of water availability (control, moderate drought, severe drought) and two temperature regimes aimed to represent contrasting field temperatures at the highest and the lowest elevation (5/2 °C and 15/7 °C, day/night).

Materials and Methods

Phacelia secunda J. F. Gmel. (Boraginaceae) is a perennial hemicryptophyte herb with a wide elevational and latitudinal distribution in Chile and Argentina. In Chile, it is distributed from c. sea level to the upper vegetation limit in the Andes, inhabiting coastal, Mediterranean-type, montane forest and alpine communities (Cavieres 2000). Plant material was collected from three elevations in the Andes of central Chile, where climate has a strong influence of the Mediterranean-type climate that prevails in the low elevations. The collection area at the low-elevation was at 1600 m a.s.l. in the private natural reserve Yerba Loca (33°10’S, 70°13’W). The mid-elevation was located at 2,900 m a. s. l. close to the La Parva Ski Center (33° 21'S, 70° 19'W) and the high-elevation collection area was at 3600 m a. s. l. (31°19’S, 70°15’W). (Cavieres et al. 2006). The low-elevation site was selected at 1600 m a.s.l. in the private natural reserve Yerba Loca (33°10’S, 70°13’W). At this elevation, the period free of snow lasts 8 months, usually starting in September and ending in April. The historical mean annual precipitation at this elevation is 445 mm, falling predominantly as rain during winter months (Santibañez and Uribe 1990). Growing season’s mean temperature at this elevation is 13.1 °C, with minimal and maximal temperatures of 10.6 and 22.5 °C, respectively (Hernández-Fuentes et al. 2015). The high-elevation site was selected at 3600 m a. s. l. (31°19’S, 70°15’W). At this elevation, the growing season lasts ca. 5 months (from December to April), and the annual precipitation is 943 mm, falling mainly as snow during the winter with occasional hail or snow during the summer (Santibañez and Uribe 1990). Thus, in contrast to the low-elevation’s site, this site has no water limitations throughout the growing season (Sierra-Almeida and Cavieres 2010). Growing season’s mean temperature at this elevation is 8.2 °C with minimal and maximal temperatures of –1.0 and 20.1 °C, respectively (Hernández-Fuentes et al. 2015).

Experimental set up

One hundred and twenty *P. secunda* plants were collected early during 2016-2017’s growing season at each elevation. We collected small seedlings (up to 4 ± 2 cm height, with 2–3 leaves) (i.e. after budburst), germinated during the same growing season of collection. Plants were collected from the field by carefully digging the soil and then extracting the complete root system. Immediately after collection, plants were placed one by one on wet absorbent paper and kept under cool conditions. Plants were transported to a greenhouse

(University of Concepción, Concepción, Chile) the day after collection. In the greenhouse, each plant was carefully transplanted into 30 cm deep pots (900 ml) filled a mixed substrate (commercial organic soil: sand, 1:1). The organic substrate (ANASAC®) had the following properties: pH (H_2O): 5.25, mineral N: 43.4 mg kg^{-1}, Olsen-P: 17.3 mg kg^{-1}, organic matter: 22.4%, base saturation: 34.7 cmol+ kg^{-1}) (Ávila-Valdés et al. 2019). Plants were irrigated twice a week and maintained for 1 month in greenhouse. After this period, during December 2017 seedlings were placed in walk-in growth chambers (PiTec S.A., Santiago, Chile) at two growth temperatures: 5/2°C day/night and 15/7°C day/night and maintained for 30 days under relative humidity average of 72%, soil moisture at field capacity, and 400 µmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) with a day-length 15 h/9 h (day/night). Temperatures used for this experiment correspond to the temperatures experienced by the plants at the beginning of the growing season (see Peñaloza et al. 2001; Reyes-Bahamonde et al. 2022). After this period, two water stress treatments were applied in both growth temperature conditions by completely suppressing irrigation until reaching the following soil water potential (Ψsoil): -1.91 ± 0.06 MPa (drought) and -3.62 ± 0.05 MPa (severe drought), while control plants were irrigated continuously and recorded -0.27 ± 0.01 MPa. Approximately, drought was reached after two weeks, while severe drought was reached after four weeks. Once the soil water potentials were reached, the plants were kept for thirty days in each condition. The Ψsoil were monitored with 12 PST-55-30 thermocouples (WESCOR, INC. West Logan, USA) installed in 2 pots for water treatments and measured every 4 days with a PSYPRO psychrometer water potential system (WESCOR, INC. West Logan, USA). Thus the experiment had six treatments, determined by two growing temperature (5/2 and 15/7°C) and three water availability.

After the experimental period, we evaluated the survival in all the plants of each elevation and treatment. In addition, six plants of each elevation and treatment were harvested to assess biomass and NSC concentrations.

Survival

Survival of individuals growing in different experimental conditions was assessed after thirty days of experiment. On the basis of visual assessment, plants were classified into two categories: healthy and living plants (Pl) or dead plants (Pd).

The survival percentage was calculated as follows:

$$\% \text{ Survival} = \frac{Pl}{Pl+Pd} x \ 100.$$

Growth

Seedlings were gently removed from pots and the roots were separated from the shoots and then washed with tap water (dead tissues attached to the plant were not excluded from the measurements). Above and underground tissue were then stored separately in paper bags, dried at 70 °C in a forced-air oven (ZXFD-B5090, LABWIT Scientific Pty Ltd., Australia) for 4 days and then weighed in an analytical balance to obtain organ-specific biomasses and total plant biomass.

Biomass differences between treatments (LRR_biomass) during the experimental period were estimated as the log10 of the ratio between the biomass of irrigated plants (control plants, b_c) and the biomass of plants under drought (b_d) and severe drought (b_{sd}) in both temperatures:

$$LRR\_biomass = \log_{10}(bd\ or\ bsd/bc),$$

Values > 0 indicate biomass increases; meanwhile values < 0 indicate biomass loss.

Carbohydrate analyses

Non-structural carbohydrate (NSC) concentrations were determined in the same plants harvested for biomass determination, for each tissue separately, as the sum of the quantitatively low molecular weight carbohydrates (*i.e.* glucose, fructose and sucrose, hereafter “total soluble sugars”: TSS) plus starch (as described in Hoch et al. (2002)). Low molecular weight sugars were extracted with distilled water at 100 °C for 60 min and analysed photometrically at 340 nm on a 96-well multiplate reader after enzymatic conversion (invertase and phosphoglucose isomerase from *Saccharomyces cerevisiae*, Sigma Aldrich I4504 and P5381, respectively, St Louis, MO, USA) of sucrose and fructose to glucose. The concentration of glucose was determined photometrically after the enzymatic conversion of glucose to gluconate-6-phosphate (Glucose Assay Reagent, G3293 Sigma Aldrich). Following the degradation of starch to glucose, using a purified fungal amylase (‘amyloglucosidase’ from *Aspergillus niger*, Sigma Aldrich 10115) at 45 °C overnight, starch was determined in a separate analysis by the same procedure. The starch concentration was calculated as NSC minus the sum of low molecular weight carbohydrates and expressed as glucose equivalents. Concentrations are presented on a percent of dry matter basis (% d.m.).

To compare the NSC concentration between treatments, the mean NSC concentration for each plant was weighted, integrating the biomass and NSC concentration of each tissue, as described in Hoch et al. (2002), using the following formula:

$$\sum_{org=1}^{n} \frac{conc_{org}\ x\ biom_{org}}{100},$$

where n is the number of organs, $conc_{org}$ is the organ-specific NSC concentration (% d. m.) and $biom_{org}$ is the organ-specific fraction of the total biomass.

Separately, fructans concentration was calculated using a method for reducing-sugars with a kit Megazyme Fructans HK Assay (AOAC Method 999.03 and AACC Method 32.32; Megazyme International Ireland Ltd., Wicklow, Ireland), following the procedure described by McCleary et al. (2000). Fructans were extracted by digesting 100 milligrams of sample in 40 ml of distilled water at 80 °C during 15 min. Two aliquots of the extract were treated with purified fructanase (fructanase; bottle 2 in Megazyme Fructans assay kit A98YV29) to hydrolyze fructans to fructose and another aliquot was treated with acetate buffer at 100 mM, pH: 4.5 (sample blank). Sugars concentration was measured at 410 nm with the para-hydroxybenzoic acid hydrazide (PAHBAH) method for reducing sugars.

To determine the fructans concentration, the following formula was used, as indicated in the kit: $\Delta A \times F \times \frac{V}{W} \times 2.48$; where, ΔA is absorbance mean between extracts treated with fructanase minus the absorbance of the blank, F is fructose factor to convert absorbance values to µg = (54.5 µg D-fructose)/(absorbance for 54.5 µg D-fructose), V is extraction volume, W is sample weight, and 2.48 corresponds to correction for all solutions during procedure. The whole-plant mean fructans concentration was estimated for each plant using the same procedure to estimate whole-plant NSC concentration.

Statistical analyses

To assess the effects of elevation, drought severity, growth temperature and the interactions among these factors on survival and LRR_biomass, we used generalized linear effects models (GLM). To assess the effects of elevation, water availability, growth temperature and the interactions among these factors on NSC concentrations and different compound (starch, fructans and TSS), we used linear effects models (GLM). Tukey honest significant difference (HSD) was used to evaluate differences between pairs of means (alpha < 0.05). LM and GLM were run in R (R 3.6.3).

Results

Survival under drought and different temperatures

Growth temperature did not significantly affect plant survival, although there was a significant interaction between temperature and elevation (significant effect of “temperature * elevation” on survival, Table 3.1). Thus, plants from 3600 m had lower survival under 15/7 °C than under 5/2 °C. The water availability had a significant effect on survival, which also depended on the elevation provenance (significant effect of “water availability * elevation” on survival, Table 3.1). In general, survival decreased with drought severity, but the decrease was steeper in plants from the highest elevation. Mean survival in plants from 1600 m was 100% under control conditions, 97.5% under mild drought, and 76% under severe drought (Fig. 3.1a). Mean survival in plants from 2800 m was 100 %, 86% and 67% for plants under control conditions, mild drought and severe drought, respectively (Fig. 3.1b). Mean survival in plants from 3600 m was 94%, for plants under control conditions, 71% for plants under mild drought and under severe drought, these plants had only 38% survival (Fig. 3.1c).

LRR_biomass

Growth temperature had significant effect on biomass, which also depended on the elevation provenance (significant effect of “temperature * elevation” on biomass, Table 3.2). Plants from 1600 and 2800 m growing at 15/7°C, had higher biomass than plants growing at 5/2°C (Fig 3.2a-b), while 3600 m plants did not show a clear pattern (Fig. 3.2c). Drought severity had a significant effect on biomass, which also depended on the elevation provenance significant effect of "water availability * elevation" on biomass Table 3.2). In general, biomass decreased with drought severity, but the decrease was steeper in plants of the highest elevation. Finally, interactions between growth

temperature, water availability and elevation had a significant effect on biomass (significant effect of "temperature * water availability * elevation" on biomass Table 3.2). Plants from 1600 m growing at 5/2 °C increased their biomass under any water availability (HSD Tukey test $p > 0.05$ for all) (Fig. 3.2a). On the other hand, plants from 1600 m growing at 15/7 °C significantly increased their biomass in all water availability (HSD Tukey test $p < 0.01$ for all), although this increase was significantly higher in control plants than in plants exposed to severe drought (Fig. 3.2a). Plants from 2800 m (Fig. 3.2b) exposed to both temperature regimes increased significantly their biomass under control conditions and mild drought (HSD Tukey test $p < 0.01$ for both), although the increase was higher in plants at 15/7 °C than in those at 5/2 °C. The biomass of these plants decreased under severe drought in both temperature regimes, but more so in the colder regime (HSD Tukey test $p < 0.01$ for both). Plants from 3600 m (Fig. 3.2c) exposed to both temperatures increased their biomass under control conditions and, to a lesser extent, under mild drought (HSD Tukey test $p < 0.01$ for both); the increase for control plants was higher in the warmer regime, while the increase under mild drought was higher in the colder regime. In addition, plants exposed to severe drought showed decreased biomass for both growth temperatures (HSD Tukey test $p < 0.01$).

Non-structural carbohydrates (NSC) concentration

Elevation had significant effect on NSC concentration (significant effect of “elevation” on NSC concentration, Table 3.3). Plants from 1600 m had higher NSC concentration on average than plants from other elevations. Mean NSC concentration in plants from 2800 m was 9% less than in plants from 1600 m (HSD Tukey test $p < 0.01$), while mean NSC concentration in plants from 3600 m was 61% less than in plants from 1600 m (HSD Tukey test $p < 0.01$. Growth temperature, had significant effect on NSC concentrations, which also depended on the elevation provenance (significant effect of “temperature * elevation” on NSC concentration Table 3.3). Generally, plants from 1600 m and 2800 m showed mean NSC concentration when growth under 15/5°C than when growth under 5/2°C (Fig. 3.3a-b), while from 3600 m showed the inverse pattern (Fig. 3.3c). The water availability had a significant effect on NSC concentration, which also depended on the elevation provenance (significant effect of “water availability * elevation” on NSC concentration Table 3.3). In plants from 1600 and 2800 m, mean NSC concentration increased slightly under mild drought and decreased under severe drought (Fig. 3.3a-b), while in plants from 3600 m, mean NSC concentration decreased slightly under both mild and severe drought (Fig. 3.3c). Finally, interaction between growth temperature, water availability and elevation had a significant effect on NSC concentration (Table 3.3). In plants from 1600 m (Fig. 3.3a) growing at 5/2 °C and mild drought, NSC concentration increased 7.2% in comparison with control plants (HSD Tukey test $p < 0.01$), while in plants growing at 5/2 °C and severe drought, NSC concentration decreased 53.3% in comparison with control plants (HSD Tukey test $p < 0.01$). In plants from 1600 m growing at 15/7 °C NSC concentration was 48.6% and 29.5% higher in plants under mild and severe drought, respectively, in comparison with control plants (HSD Tukey test $p < 0.01$ for all). In plants from 2800 m (Fig. 3.3b) at 5/2 °C, NSC concentration increased 85% under mild drought in comparison with control plants (HSD Tukey test $p < 0.01$), while under severe drought NSC concentration did not differ with control plants (HSD Tukey test $p > 0.05$). In plants from 2800 m at 15/7 °C, NSC concentrations were 48.3% and 28.6% higher under mild and severe drought, respectively, in comparison with control plants (HSD Tukey test $p < 0.01$ for all). Finally, in plants from 3600 m (Fig. 3.3c) NSC concentrations decreased with the drought severity, and more so under 15/7 °C. At 5/2 °C and mild drought, NSC concentration decreased by 35.5% and 78.5% under mild and severe drought, respectively, in comparison with control plants (HSD Tukey test $p < 0.01$ for both). At 15/7 °C, NSC concentrations in plants from 3600 m decreased by 42.7% and 92.8% under mild and severe drought, respectively, in comparison with control plants (HSD Tukey test $p < 0.01$ for all).

Starch, fructans and Total Soluble Sugar

Elevation had significant effect on starch, TSS and fructans concentration (significant effect of “elevation” on starch, TSS and fructans, Table 3.4a-c, repectively). On average, plants from 1600 m and 2800 had higher starch and TSS concentration than plants from 3600 m. But, plants from 2800 m had less fructans concentration than plants from 1600 m and 3600 m.

Water availability, elevation and growth temperature had effect on starch, TSS and fructans concentration (significant effect of “water availability * elevation * growth temperature” on starch, TSS and starch, Table 3.4a-c). Plants from 1600 m (Fig. 3.4a) increased all NSC compounds under mild drought respect to control plants (HSD Tukey test $p < 0.001$ for all). However, this increase was high for TSS concentration than fructans and starch concentrations. On the other hand, under severe drought, plants from 1600 m (Fig. 3.4a) showed different response depending to growth temperature. Plants exposed to 5/2°C decreased all NSC compounds respect to control plants (HSD Tukey test $p < 0.001$), inclusive fructans concentrations decreased to 0 (HSD Tukey test $p < 0.001$). In plants exposed to 15/7°C TSS concentration increased (HSD Tukey test $p < 0.001$), but fructans concentrations remains unchanged with respect to control plants (HSD Tukey test $p > 0.05$). Plants from 2800 m (Fig. 3.4b) under mild drought, showed different response depending to growth temperature. Plants exposed to 5/2°C increased all NSC compounds respect to control plants (HSD Tukey test $p < 0.001$ for all). In plants exposed to 15/7°C TSS and starch concentration increased (HSD Tukey test $p < 0.001$ for both), but fructans concentrations decreased with respect to control plants (HSD Tukey test $p < 0.001$). Likewise, under severe drought, plants from 2800 m (Fig. 3.4b), also showed different response depending to growth temperature. In plants exposed to 5/2°C starch concentration decreased (HSD Tukey test p

< 0.001) and TSS concentration remains unchanged with respect to control (HSD Tukey test $p > 0.05$). In plants exposed to 15/7°C TSS and starch concentration increased (HSD Tukey test $p < 0.001$), but fructans concentrations decreased to 0 with respect to control plants (HSD Tukey test $p < 0.001$). Finally, in plants from 3600 m (Fig. 3.4c) under mild and severe drought, all NSC compounds decreased (HSD Tukey test $p < 0.001$ for all). However, this decreased was high in plants exposed to 15/7°C than 5/2°C.

Discussion

Our study shows that *Phacelia secunda* plants naturally exposed to drier and warmer conditions (i.e. low elevation), are more drought resistant than their counterparts inhabiting more humid and colder conditions (i.e. high elevation). Additionally, we demonstrate that the dynamics of NSC storage in response to drought differed between elevations, and suggest a mechanistic explanation. Drought caused a progressive NSC reduction in high elevation plants, with NSC decreasing up to critical levels under severe drought conditions. By contrast, in mid- and low- elevation plants, severe drought caused only a slight NSC reduction and under the lowest temperature only, while mild drought caused NSC to increase. Additionally, there was a significant sugar accumulation in 1600 m and 2800 m plants in response to drought, particularly for the warmer treatment. These results are in line with the immediate role of NSC, i.e. in osmoregulation (Martínez-Vilalta et al. 2016), particularly in drought-adapted plants. Soluble sugars decrease the cell osmotic potential and consequently can increase the cell turgor (Hartmann et al. 2021; Munns and Weir 1981). The capacity of plants to keep a high (i.e. more negative) osmotic potential, either under full turgor (i.e. constitutive osmotic potential) or at the point of turgor loss, is related to the species drought tolerance (Bartlett et al. 2012). Although starch does not play an osmotic role, it is thought that plants need to keep a permanent starch reservoir in order to account for a readily available source of sugars (Sapes et al. 2021, Martinez Vilalta et al. 2016). Accordingly, C-starved plants exhibit a higher (less negative) osmotic potential and are more vulnerable to cavitation, even under well-watered conditions (Tomasella et al. 2021, Sapes et al. 2021). Thus, we suggest that the higher NSC and TSS concentrations of low and mid elevation plants, in comparison to high elevation plants, reflect a higher constitutive osmotic potential and the capacity to perform osmotic adjustment under drought (Hartmann et al. 2021). Such a capacity would be beneficial under the drier conditions typically characterizing the lower elevations.

Temperature had contrasting effects on NSC concentration, depending on the elevation and the drought level. Low elevation plants exposed to severe drought increased the NSC concentration only under 15/7°C. However, under 5/2°C, NSC concentration was still higher than the NSC concentration of high elevation plants. The temperature-dependent NSC responses to drought in lower elevation plants could be explained by differences in the osmotic compounds. Thus, we suggest that *P. secunda* requires different type of osmotic compounds to resist drought at low versus high temperature. Compounds as sugar alcohols or amino acids (proline and ectoine) (Singh et al. 2015), which we did not measure, could have been increased under severe drought and low temperatures, conferring cell turgor and eventually explaining the high survival recorded in these plants. As carbohydrates are precursors of these compounds, and as photosynthesis was reduced by drought, their synthesis might have to rely on the NSC pool. This could explain why NSC concentration was reduced under drought and low temperature. Another possible explanation for the NSC concentration's decrease in low elevation plants exposed to drought and low temperature is that C is moving towards a sink that we have not evaluated, such as root exudates or mycorrhization (see Karst et al. 2017; Sapes et al. 2021). However, we are not aware of increasing C exportation to roots under colder conditions, and this is therefore an area that deserves further exploration. On the other hand, high elevation plants exposed to mild and severe drought presented decreasing NSC concentration with drought, under the two growing temperatures. The greater survival sensitivity to drought in high elevation plants could be due to insufficient sugar availability requeried for osmotics. Indeed, NSC concentrations presented by high elevation plants were almost depleted under the warmer treatment, in big contrast to what we found in low elevation plants. If so, high elevation plants could have been unable to maintain tissue turgor when exposed to experimental drought conditions. Alternatively, low NSC levels resulted insufficient to keep metabolic demands under the warmer condition, where respiration is expected to be greater. More studies are needed to evaluate the mechanism by which the lack of NSC triggers high mortality in higher elevation plants exposed to drought.

The dynamics of different NSC compounds under drought was consistent with differences in drought tolerance between elevations. Low and mild elevation plants increased all compound under mild drought, while high elevation plants decreased all compound under mild drought. In low elevation plants exposed to mild drought, TSS was the compound that increased more than others. The greater TSS concentration in conditions of low water availability, could be an intrinsic response of low-elevation plants, since they are naturally subjected to dry and warm summers (Hernández-Fuentes et al. 2015). Our results agree with those of Garcia et al. (2011), who found that *Laretia acaulis*, an alpine cushion plant from central Chile, had high concentrations of low-weight molecular sugars at low and mid-elevations throughout the growing season, which could reflect a high C investment in osmoregulation and osmoprotection. Our results are consistent with the role that NSCs play under drought conditions in plants that are naturally under drought. The high concentrations of NSC found in plants at lower elevations, plus the increase in concentrations under drought conditions, suggest that there is an immediate role for NSCs in plants that are naturally exposed to drought, which is not the case in plants at higher elevations. Our study demonstrates that plants naturally exposed to drought (low elevation) are more resistant to drought than those that do not naturally experience drought.

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Tables

Table 3.1 Results from generalized linear models (GLM) testing the effects of elevation (1600, 2800 and 3600 m a.s.l), water availability (drought and several drought) and temperature (5/2°C and 15/7°C) and the interactions among these factors on the survival, in the herbaceous alpine plant *Phacelia secunda* from the Mediterranean Andes of Central Chile (Fig. 3.1).

	Df	Chisq	Pr(<Chisq)
Survival			
Elevation (E)	2	56.415	8.03e-12 ***
Water availability (WC)	2	217.935	< 2e-16 ***
Temperature (T)	1	2.006	0.1653
E*WC	4	10.32	1.13e-05 ***
E*T	2	12.261	8.69e-05 ***
WC*T	2	3.074	0.0585
E*WC*T	4	1.312	0.2842

Table 3.2 Results from generalized linear models (GLM) testing the effects of elevation (1600, 2800 and 3600 m a.s.l), water availability (drought and several drought) and temperature (5/2°C and 15/7°C) and the interactions among these factors on the LRR_biomass, in the herbaceous alpine plant *Phacelia secunda* from the Mediterranean Andes of Central Chile (Fig. 3.2).

	Df	Chisq	Pr(<Chisq)
LRR_biomass			
Elevation (E)	2	136.937	< 2e-16 ***
Water availability (WC)	2	243.111	< 2e-16 ***
Temperature (T)	1	24.75	3.11e-06 ***
E*WC	4	42.238	< 2e-16 ***
E*T	2	7.734	0.000796 ***
WC*T	2	1.178	0.31252
E*WC*T	4	0.0199	0.005685 **

Table 3.3 Results from a variance analysis (ANOVA) testing the effects of elevation (1600, 2800 and 3600 m a.s.l), water availability (drought and several drought) and temperature (5/2°C and 15/7°C) and the interactions among these factors on the concentrations of non-structural carbohydrates (NSC) in the herbaceous alpine plant *Phacelia secunda* from the Mediterranean Andes of Central Chile (Fig. 3.3).

	Df	F-value	Pr(<F)
NSC			
Elevation (E)	2	285.33	< 2e-16 ***
Water availability (WC)	2	82.96	< 2e-16 ***
Temperature (T)	1	10.52	0.00166 **
E*WC	4	38.88	< 2e-16 ***
E*T	2	19	1.30e-07 ***
WC*T	2	20.07	6.19e-08 ***
E*WC*T	4	13.04	2.03e-08 ***

Table 3.4 Results from a variance analysis (ANOVA) testing the effects of elevation (1600, 2800 and 3600 m a.s.l), water availability (drought and several drought) and temperature (5/2°C and 15/7°C) and the interactions among these factors on the concentrations of compounds (starch, fructans, and total low molecular weight sugars) in the herbaceous alpine plant *Phacelia secunda* from the Mediterranean Andes of Central Chile (Fig. 3.4).

Factor	Df	F-value	Pr(<F)
(a) Starch concentration (%DM)			
Elevation (E)	2	73.274	<0.001***
Water availability (WC)	2	13.919	<0.001***
Temperature (T)	1	0.423	0.517
E*WC	4	4.960	0.001**
E*T	2	1.972	0.145
WC*T	2	8.493	<0.001***
E*WC*T	4	2.515	0.047*
(b) TSS concentration (%DM)			
Elevation (E)	2	73.956	<0.001***
Water availability (WC)	2	15.949	<0.001***
Temperature (T)	1	53.825	<0.001***
E*WC	4	12.431	<0.001***
E*T	2	21.014	<0.001***
WC*T	2	1.311	0.275
E*WC*T	4	3.401	0.012
(c) Fructans concentration (%DM)			
Elevation (E)	2	36.18	<0.001***
Water availability (WC)	2	170.16	<0.001***
Temperature (T)	1	19.88	<0.001***
E*WC	4	31.755	<0.001***
E*T	2	5.023	0.009
WC*T	2	2.155	0.122
E*WC*T	4	1.768	0.142

Figures

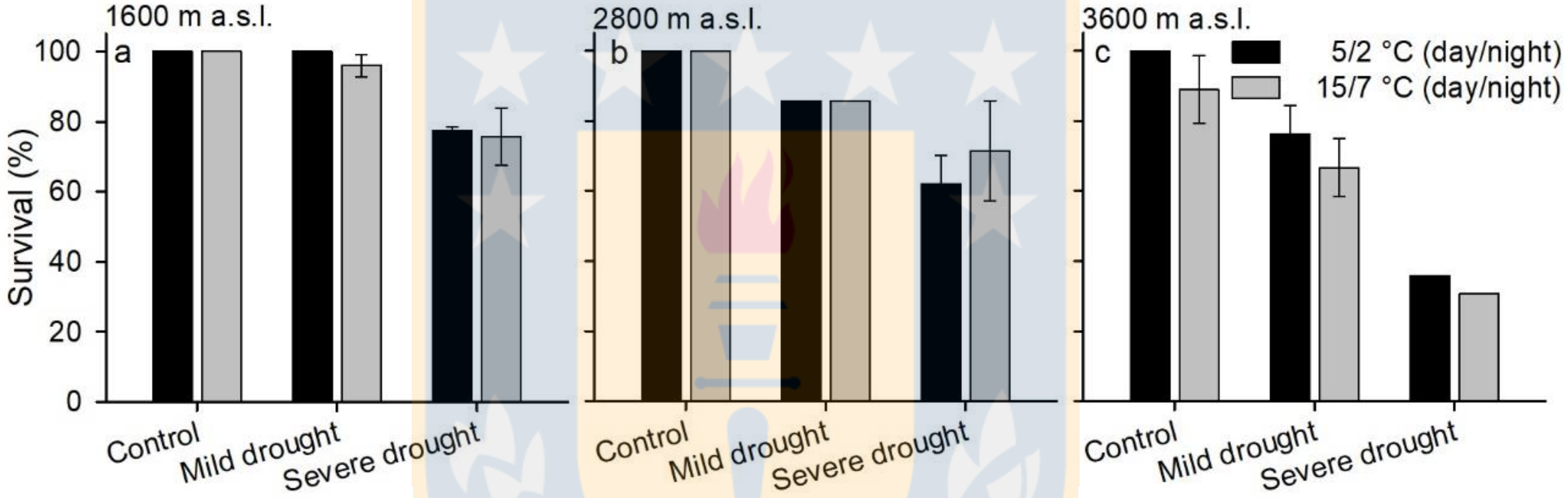

Figure 3.1 Survival percentage of herbaceous alpine plant *Phacelia secunda* from three elevations of the Mediterranean Andes of Central Chile: 1600 m (a), 2800 m (b) and 3600 m (c) growing at two temperatures: 5/2°C (black bars) and 15/7 °C (gray bars) and three different water availability (control, drought and severe drought). Error bars denote ± 2SE of the mean (n=3 blocks).

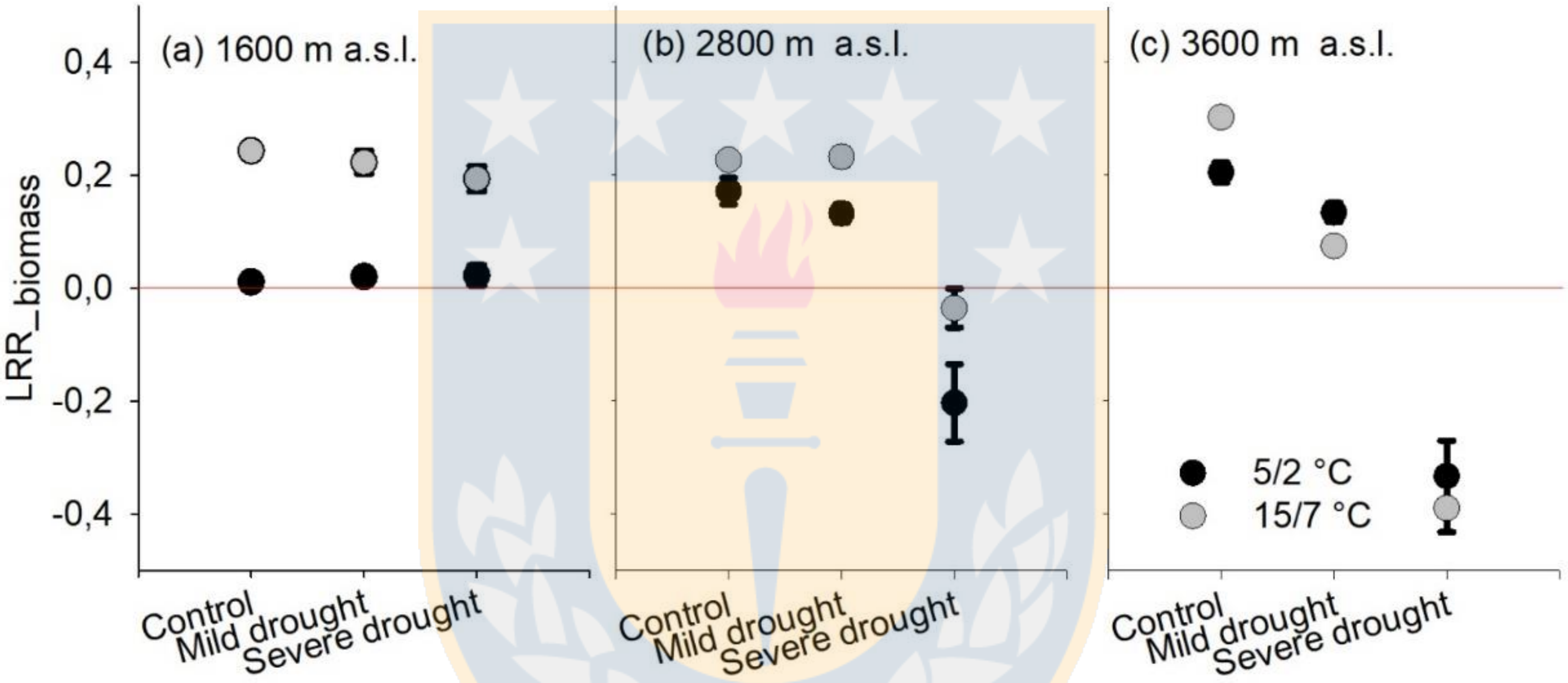

Figure 3.2 LRR_biomass of herbaceous alpine plant *Phacelia secunda* from three elevations of the Mediterranean Andes of Central Chile: 1600 m (a), 2800 m (b) and 3600 m (c) growing at two temperatures: 5/2°C (black circle) and 15/7 °C (gray circle) and three different water availability (control, drought and severe drought). Error bars denote ± 2SE of the mean (n=6).

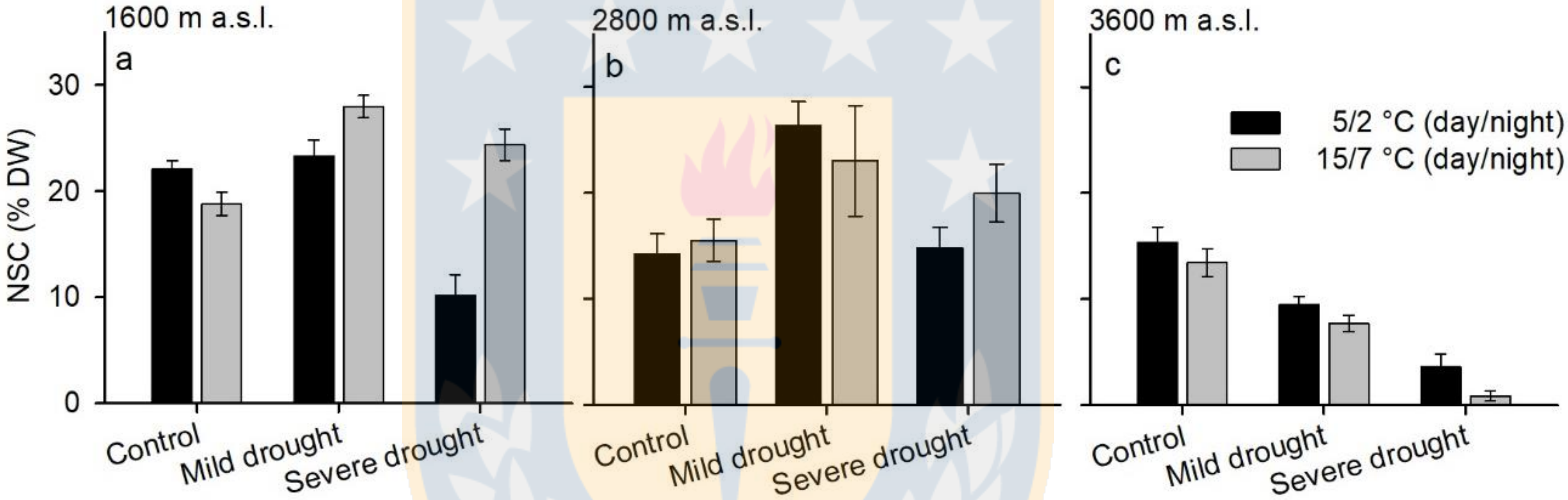

Figure 3.3 Nonstructural carbohydrates (%DW) of herbaceous alpine plant *Phacelia secunda* from three elevations of the Mediterranean Andes of Central Chile: 1600 m (a), 2800 m (b) and 3600 m (c) growing at two temperatures: 5/2°C (black bars) and 15/7 °C (gray bars) and three different water availability (control, drought and severe drought). Error bars denote ± 2SE of the mean (n=6).

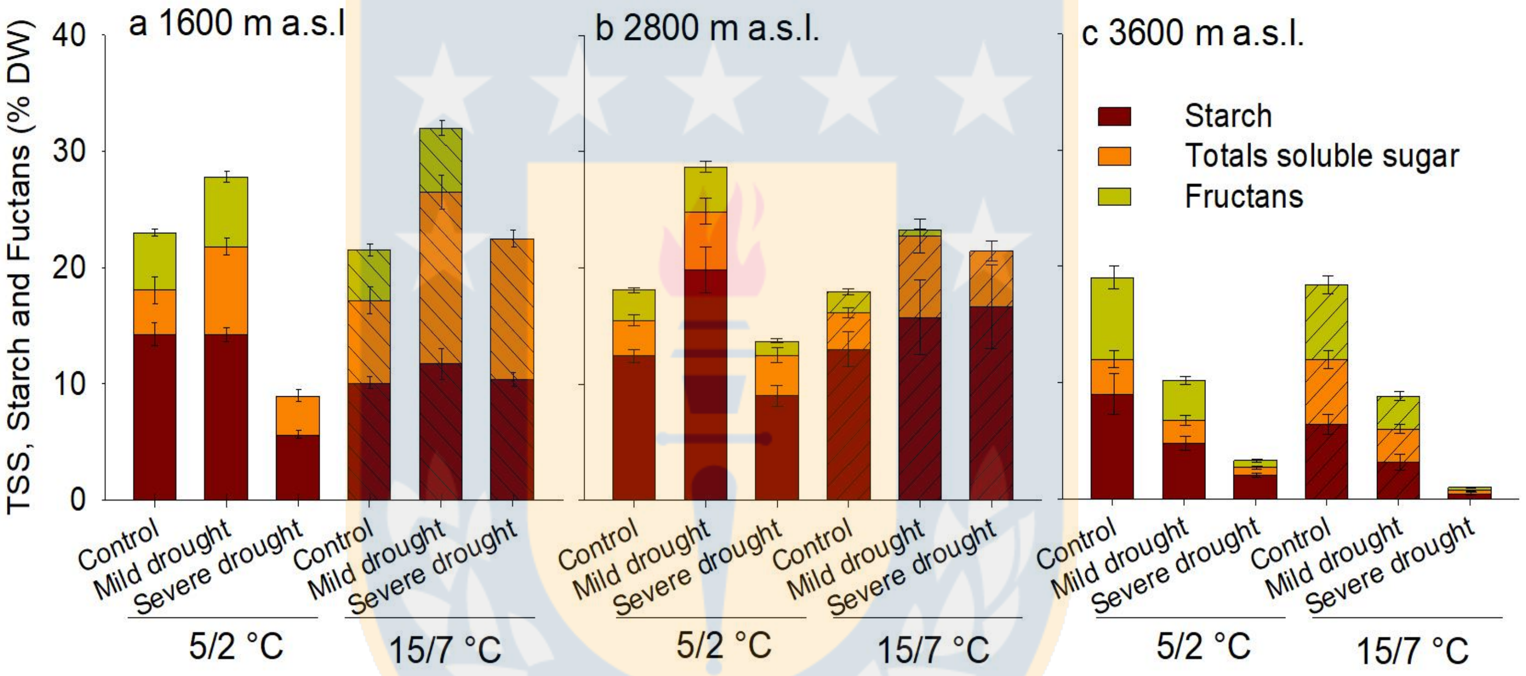

Figure 3.4 Concentrations of starch, fructans and total soluble sugars (TSS) in herbaceous alpine plant *Phacelia secunda* from three elevations of the Mediterranean Andes of Central Chile: 1600 m (a), 2800 m (b) and 3600 m (c) growing at two temperatures (5/2°C and 15/7 °C) and three different water availability (control, drought and severe drought). Error bars denote ± 2SE of the mean (n=6).

DISCUSIÓN GENERAL

Las características particulares que definen a los ambientes de montaña, como alta radiación, disminución de temperatura con la elevación, y breve período libre de nieve, han sido prevalecientes en el estado del arte de los rasgos y mecanismos que permiten la vida de las plantas que allí habitan. Se ha descrito que las plantas de alta montaña presentan características morfológicas y fisiológicas que les permiten el establecimiento y desarrollo en estos ambientes (Billings 1974; Bliss 1971; Körner 2003a; Mooney and Billings 1965), y que tanto la supervivencia en el largo período invernal como el crecimiento vegetativo al inicio de la estación de crecimiento dependen del almacenamiento de C no estructurales (Körner et al. 2019). En la misma línea, se ha planteado que el aumento de temperatura global asociado a cambio climático afectará la asignación del C en las plantas alpinas, promoviendo mayor crecimiento (Kudernatsch et al. 2008) y eventualmente cambiando su distribución hacia elevaciones mayores (Inouye 2020). Sin embargo, una mayor asignación de C a biomasa podría significar una menor asignación a almacenamiento (Blumstein et al. 2020, Dietze et al. 2014), afectando negativamente la supervivencia.

Considerando que en regiones con clima mediterráneo las plantas de alta montaña son también afectadas por sequía, en esta tesis investigamos el rol de la sequía en la asignación del C a almacenamiento y a crecimiento, en la especie *P. secunda* en el gradiente altitudinal de los Andes de Chile central. Al igual que en las regiones montañosas de clima templado, las montañas de regiones con clima mediterráneo presentan características tales como alta radiación, disminución de temperatura con la elevación, y disminución altitudinal del ya breve período libre de nieve. Sin embargo, los ecosistemas de montaña de regiones con clima mediterráneo presentan además un largo período de sequía que ocurre durante la estación estival, y que coincide con la breve temporada de crecimiento de la vegetación en estos hábitats (Cavieres et al. 2000; Körner 2003a). La sequía estival en estos ecosistemas se intensifica hacia elevaciones menores, dado que la precipitación aumenta con la elevación (Piper et al. 2016). Por lo tanto, el gradiente altitudinal de montañas con clima mediterráneo incluye dos gradientes de estrés que actúan en direcciones opuestas: por un lado, el estrés por baja temperatura aumenta con la altitud, y por el otro, el estrés por sequía se intensifica a medida que disminuye la altitud (Pescador et al. 2015). A pesar de que la sequía ejerce un control predominante en el uso y la asignación de C en plantas, y que limita la supervivencia y la distribución de éstas, su rol en las adaptaciones de plantas alpinas ante escenarios climáticos presentes y futuros ha sido escasamente considerado (Magaña Ugarte et al. 2019). Producto del cambio climático ésta se ha intensificado causando significativos cambios comunitarios y ecosistémicos (ej. menos secuestro de C producto de mortalidad de bosques). Para poder predecir las respuestas al cambio climático por parte de plantas alpinas de regiones con clima mediterráneo, es necesario identificar los mecanismos fisiológicos que resultan fundamentales para la supervivencia de éstas. Aunque se sabe que las plantas alpinas en general dependen del almacenamiento de C para cumplir su ciclo de vida, se desconocen los efectos de la sequía sobre la dinámica del almacenamiento de C.

La estación de crecimiento en plantas alpinas de ambientes sin restricciones hídricas está determinada por la temperatura (Billings 1973). Hacia mayores elevaciones, la diminución del período de temperatura favorable para el crecimiento y la ganancia de C, implica, además, períodos desfavorables (invierno) más largos. En el invierno las plantas de montaña permanecen bajo una capa de nieve, sobreviviendo únicamente a expensas de sus reservas de C (Fonda and Bliss 1966), lo que ocurriría en menor intensidad en plantas de menor elevación, debido a que el período de temperatura desfavorable es más corto. Sin embargo, dados las gradientes de sequía y temperatura que caracterizan a los gradientes altitudinales de clima mediterráneo, el almacenamiento de C podría ser fundamental para resistir la sequía, permitiendo a las plantas de menor elevación sobrevivir esta condición. Así, la primera hipótesis de esta tesis establece que el máximo estacional de NSC ocurre más tempranamente en menores elevaciones, y que la temporada de crecimiento en plantas de menor elevación es tan breve como en elevaciones más altas (capitulo 1). Para poner a prueba esta hipótesis, se colectaron mensualmente individuos de *P. secunda* de dos elevaciones contrastantes y se midió la biomasa y el almacenamiento de C. Los resultados mostraron que la máxima concentración de NSC y la mayor tasa de formación de biomasa ocurrieron dos meses antes en plantas de 1600 m que en plantas de 3600 m (Figura 1.3 y 1.4 capitulo 1). Estos resultados demuestran que la dinámica estacional de crecimiento y NSC difiere significativamente entre elevaciones no sólo debido a la disminución altitudinal de temperatura sino también debido a la influencia de la sequía. Los resultados además demuestran que el largo de la estación de crecimiento es restringido por la sequía en elevaciones menores. El máximo nivel de NSC en la menor elevación fue encontrado inmediatamente antes de la ocurrencia de sequía, mientras que en los meses de sequía más severa la concentración de NSC se mantuvo o incluso decreció junto con la conductancia estomática y fotosíntesis, demostrando que la re-movilización de C no estructural contribuyó a cubrir requerimientos metabólicos durante el período más cálido y seco. La disminución significativa de NSC observada en *P. secunda* a menor elevación durante el segundo año de mediciones se debió enteramente a una disminución de almidón; los azúcares solubles y los fructanos se mantuvieron e incrementaron, respectivamente, su concentración. Dado que tanto los azúcares como los fructanos cumplen roles de osmorregulación (Taiz and Zeiger 2003), este resultado sugiere que no sólo los requerimientos metabólicos sino también los osmóticos fueron cubiertos mediante los NSC. Magaña Ugarte et al. (2019) encontraron que varios azúcares solubles y la prolina incrementaron durante el período seco en 7 especies alpinas en el sur de España, indicando que la regulación osmótica durante el verano es un importante sumidero de C en plantas alpinas sujetas a sequía. Por lo tanto, se concluye que la sequía anticipa el máximo de almacenamiento de C de *P. secunda*, y se sugiere que dicha anticipación es ventajosa para cubrir las demandas metabólicas y osmóticas del período estival e invernal.

Los altos niveles de almacenamiento de C que observados hacia finales de la estación de crecimiento en plantas de *P. secunda* de ambas elevaciones, se condicen con la dinámica de NSC observada en especies que habitan ambientes caracterizados por baja temperatura y baja disponibilidad hídrica. Sin embargo, existe discusión respecto a si estos altos niveles de NSC reflejan una acumulación pasiva debido a una disminución más pronunciada en las demandas de C para el crecimiento que en la ganancia del C (Chapin et al. 1990; Körner 2003a), o si reflejan un cambio en la prioridad de asignación del C desde el crecimiento al almacenamiento (Dietze et al. 2014; Sala et al. 2012; Wiley and Helliker 2012). Para determinar si el almacenamiento de NSC en individuos de baja y alta elevación es una prioridad de asignación de C relativa al crecimiento, se realizó un experimento de invernadero exponiendo individuos de ambas elevaciones a un período de sombra severa y así generando una condición de agotamiento de C. Posteriormente los individuos fueron expuestos nuevamente a la luz, midiéndose la asignación de C a biomasa y a almacenamiento. Los resultados mostraron que individuos de ambas elevaciones priorizaron el almacenamiento respecto a la formación de biomasa, pero esta priorización ocurrió antes y fue de mayor magnitud en los individuos de *P. secunda* de altas elevaciones. El hecho que tanto las plantas que experimentan sequía (baja elevación) como las que experimentan temperaturas muy bajas (alta elevación) hayan priorizado el almacenamiento respecto al crecimiento sugiere que las concentraciones típicamente altas que caracterizan a las plantas de alta montaña son adaptativas y no sólo el resultado de acumulación dado por la mayor sensibilidad del crecimiento que la fotosíntesis a las bajas temperaturas o sequía. Una priorización de almacenamiento relativa al crecimiento ha sido encontrada también en árboles que han sido completamente defoliados (Piper et al. 2015) y en plántulas de especies arbóreas sujetas a condiciones experimentales similares a las de este estudio (Weber et al. 2018). Se ha propuesto que la asignación prioritaria hacia almacenamiento sería parte de una estrategia preventiva ante escenarios de agotamiento de C, como podría ocurrir en condiciones de sequía severa y prolongada, o en ambientes con alta frecuencia de disturbios y riesgo de defoliación (Wiley and Helliker 2012). En el caso de *P. secunda*, la priorización de almacenamiento en individuos de alta elevación, podría ser ventajosa para sobrevivir frente a disturbios impredecibles que afectan negativamente la ganancia de C, tales como heladas tardías o nevadas estivales (Greenland and Losleben 2001). En individuos de baja elevación podría ser ventajosa para resistir la sequía estival, cubriendo requerimientos metabólicos y osmóticos vía re-movilización del C almacenado (ver capítulo 3). Se ha observado que bajas concentraciones de NSC en plantas sombreadas, con y sin sequía, perjudican la osmorregulación, el mantenimiento de la turgencia e impiden la recuperación hidráulica (Sapes et al. 2021; Tomasella et al. 2021). No obstante, y si bien recientemente se ha demostrado que la asignación de C a almacenamiento compromete la asignación a crecimiento (Blumstein et al. 2022), aún no está claro si la asignación a almacenamiento es adaptativa.

La priorización de asignación de C a almacenamiento podría ser ventajosa para resistir la sequía estival. Es por ello que, en esta tesis, se propuso evaluar esta hipótesis. Para determinar si la asignación de C a almacenamiento en *P. secunda* de los Andes de Chile Central de baja elevación es ventajosa para sobrevivir en condiciones de sequía, se realizó un experimento de invernadero exponiendo individuos de tres elevaciones a un período de diferentes condiciones hídricas (sequía moderada y severa) bajo dos temperaturas de crecimiento (15/7 y 5/2°C). Al cabo del período de experimentación, se midieron la supervivencia, biomasa y concentraciones de NSC. Los resultados mostraron que las plantas de *P. secunda* de elevaciones menores, son más resistentes a la sequía que aquellas de mayor elevación. Individuos de menor elevación presentaron mayor supervivencia, la cual estuvo asociada a altos contenidos de NSC en comparación con plantas mayor elevación. Una mayor supervivencia bajo condiciones de sequía, conferido por mayor contenido de NSC, ha sido observado en estudios realizados principalmente en árboles, (e.g. O'Brien et al. 2014). En nuestras plantas, la sequía provocó una reducción de NSC en las plantas de mayor elevación, llegando a niveles críticos bajo sequía severa. Por el contrario, en las plantas de altura media y baja, la sequía severa causó solo una ligera reducción de NSC, mientras que la sequía leve provocó un aumento de NSC. Estos resultados están en línea con el papel inmediato de NSC, es decir, en la osmorregulación (Martínez-Vilalta et al. 2016), particularmente en plantas adaptadas a la sequía. Por ello, sugerimos que concentraciones más altas de NSC y TSS de las plantas de media y baja elevación, en comparación con las plantas de mayor elevación, reflejan un mayor potencial osmótico constitutivo y la capacidad de realizar un ajuste osmótico en condiciones de sequía

(Hartmann et al. 2021). Tal capacidad sería beneficiosa bajo las condiciones más secas y cálidas que típicamente caracterizan las elevaciones más bajas. Así, este estudio, aún con sólo una especie como modelo, generó conocimiento sobre el rol que cumplirían los NSC en plantas de baja elevación en ambientes alpinos con influencia mediterránea.

CONCLUSIONES GENERALES

Los resultados obtenidos a partir de los estudios realizados durante el desarrollo de esta tesis (Fig. 4), mostraron que plantas de *P. secunda* de elevaciones contratantes parecen ser diferentes ecotipos, y que las principales diferencias en cuanto a su crecimiento y almacenamiento de NSC, está dado por la particularidad ambiental a la que están expuestas naturalmente (sequía estival en baja elevación y bajas temperaturas en alta elevación). La dinámica estacional de crecimiento y almacenamiento de NSC en plantas de menor elevación alcanzó su máximo a mediados de verano, junto con la disminución de la fotosíntesis y disponibilidad hídrica, debido a la sequía estival. Por el contrario, la dinámica estacional de crecimiento y almacenamiento de NSC en plantas de mayor elevación alcanzó su máximo hacia principios de otoño, junto con la disminución de la fotosíntesis y temperatura ambiental. Si bien las plantas de ambas elevaciones priorizaron el almacenamiento en desmedro del crecimiento, esto fue evidentemente mayor para plantas de elevaciones altas en comparación con las de elevaciones bajas, lo que podría reflejar un importante rol de los NSC en las plantas de mayor elevación. Este rol puede estar dado por las condiciones ambientales a las que están naturalmente sometidas éstas plantas (*e.g* heladas estivales). No obstante, sólo los individuos de menor elevación fueron capaces de sobrevivir de mejor manera a un período de sequía y sequía severa. Lo anterior, se correlacionó positivamente a las concentraciones de NSC que las plantas presentaron. Plantas de menor elevación presentaron alta supervivencia y altas concentraciones de NSC, mientras que plantas de alta elevación presentaron bajas concentraciones de NSC y baja supervivencia. Es así que, bajo escenarios de disminución en la disponibilidad hídrica, aunque el almacenamiento de C sea prioritario en plantas de mayor elevación, éstas no serían capaces de mantener sus reservas y por lo tanto podrían presentar mayor mortalidad. Todo lo anterior tiene fuerte implicancias en las posibles respuestas de las plantas de esta especie al cambio climático. En un escenario donde la disponibilidad hídrica se vea afectada por cambios en las precipitaciones, baja acumulación de nieve o por el acelerado deshielo de ésta, la supervivencia de las poblaciones de *P. secunda* de alta elevación, podría verse afectada.

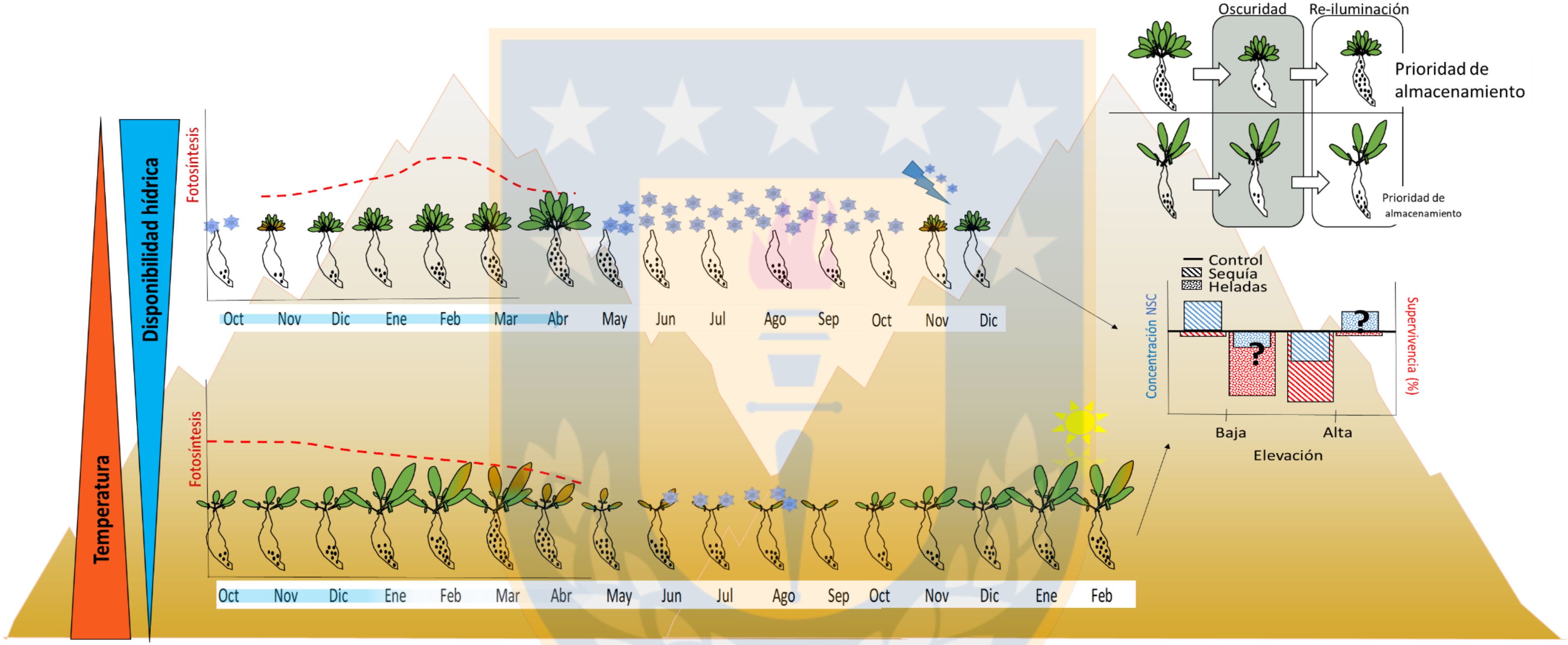

Figura 4 Resumen gráfico de resultados obtenidos en esta tesis. Dinámica estacional de NSC y crecimiento: Las concentraciones máximas de NSC en 1600 m, se encontraron hacia mediados del verano junto con el cese de crecimiento. Por el contrario, a 3600 m se observaron en otoño junto con el cese del crecimiento. Prioridad de asignación: El período de re iluminación provocó un mayor aumento en la concentración de NSC que en la biomasa en las plantas de baja y alta elevación, pero ésta fue mucho más intensa para las plantas de mayor elevación. ¿NSC confieren supervivencia bajo sequía?: Plantas de menor elevación presentaron mayor supervivencia frente a sequía, la cual estuvo asociada a mayor concentración de NSC.

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