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ORIGINAL PAPER

Defoliation reduces growth but not carbon reserves in Mediterranean *Pinus pinaster* trees

Elena Puri[1] · Günter Hoch[1] · Christian Körner[1]

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Abstract
Key message **Reduced growth but high NSC after severe defoliation of evergreen trees can be explained by three, non-exclusive processes: critical loss of non-C reserves, hormonal changes, and prioritisation of C storage over growth.**
Abstract In an attempt to simulate processionary moth impact on pines, we explored the extent to which late winter defoliation affects growth and carbon reserves in the following season. In separate treatments we removed 100, and 50 % of needles of whole trees and defoliated single branches in naturally grown, 2–3-m-tall *Pinus pinaster* trees in Italy. Shoot and stem growth (lateral shoot length and basal area increment, respectively) were substantially reduced after 100 % defoliation (−45 % for shoots, −84 % for stems). In 50 % defoliated trees only stem growth was reduced (−37 %), and in trees with a single branch defoliated, growth remained unaffected. Although substantial carbon and nitrogen reserves were removed from defoliated trees prior to bud break, non-structural carbohydrates (NSC) concentrations in branches and needles fell below control values only during the first half of the growing season, and considerable amounts of NSC persisted throughout the year. By the end of the dry and hot Mediterranean summer, NSC concentrations in branch xylem, branch phloem, previous year needles, stem sapwood and root xylem were similar among all treatments. Reduced growth and high late season NSC after defoliation can be explained by (1) a critical loss of reserves other than C (e.g. N and P), (2) hormonal changes which affected cambial activity, or (3) a prioritisation of carbon storage over growth, with all three mechanisms potentially contributing to the observed growth and NSC response.

Keywords Carbon limitation · Conifers · Herbivory · Non-structural-carbohydrates · Starch · Storage · Sugars

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✉ Günter Hoch
guenter.hoch@unibas.ch

[1] Institute of Botany, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland

Introduction

Defoliation by insect herbivores represents a great disturbance for trees. A defoliated tree can compensate for the loss of foliage by producing new shoots and leaves. Because net carbon (C) gain by current photosynthesis is greatly reduced after foliage is lost, the balance between C sinks and sources is altered and growth of new shoots will, at least initially, depend upon the mobilisation of stored reserves. Chapin et al. (1990) identified three storage processes for reserves in plants: (1) accumulation (i.e. increase in compounds due to a periodic overproduction compared to the demand for structural growth), (2) reserve formation (i.e. the synthesis of compounds for storage that could otherwise directly feed into growth) and (3) recycling (reutilisation of compounds). Non-structural carbohydrates (NSC) are defined as the sum of free, low molecular weight sugars and starch, and serve as the primary storage compounds for trees, although lipids are also important for some genera including *Pinus* (Hoch et al. 2003; Körner 2003). NSC concentrations in plants are generally assumed to reflect the difference between C uptake (photosynthesis; source activity) and C demand (metabolism, growth and export; sink activity) and are therefore considered to be

indicative of the organisms' C supply status (Körner 2003; Hoch 2015). C reserves accumulate during periods of C surplus, and can later be mobilised to supply structural growth when C demand exceeds available C from source activity, e.g. after defoliation (Chapin et al. 1990). Depending on the magnitude of the C demand, the growth of trees may become C source (photosynthesis) limited. Hypothetically, plants might prioritise C reserve formation over the demand for structural growth. One such situation might be the defoliation of evergreen tree species (Wiley et al. 2014). Whenever climatic driven growth restrictions in trees occur (i.e. during drought and critically low temperature), physiological evidence clearly suggests a higher sensitivity of growth than of photosynthesis, often resulting in an accumulation of photoassimilates (Palacio et al. 2014). However, in cases where C supply is eliminated, such as after defoliation, C reserves must operate as the sole C source in plants until new leaves are rebuilt.

Several previous studies demonstrated that defoliation of evergreen trees can lead to significant reductions in carbohydrate reserves in leaves (Ericsson et al. 1980, 1985), roots and stems (Li et al. 2002; Jacquet et al. 2014), and whole seedlings (Vanderklein and Reich 1999). Similarly, in deciduous trees, defoliation can lead to a reduction in carbohydrates in roots (Wargo et al. 1972; Kolb et al. 1992) and stems (Tschaplinski and Blake 1994). Only recently, Piper and Fajardo (2014) showed that the NSC decline following 50 % defoliation was higher in an evergreen compared to a deciduous *Nothofagus* species in leaf, stem and root tissues. However, several studies have also found no effect of defoliation on carbohydrate reserves in evergreen (Handa et al. 2005; Barry et al. 2011) and deciduous trees (Raitio et al. 1994).

In the Mediterranean region, the pine processionary moth (*Thaumetopoea pityocampa* Dennis & Schiff.) is an important insect defoliator. The caterpillars of these moths feed at night on previous season foliage of *Pinus* and *Cedrus* species during late winter and early spring, with the peak activity commonly between February and March (Carus 2004). Higher winter temperatures and fewer frost days lead to increased survival and feeding, and warmer climate conditions can allow previously unsuitable sites to become accessible (Bale et al. 2002). In fact, various authors have reported a substantial recent increase in the elevation and latitude of the pine processionary moth distribution area (Goussard et al. 1999; Battisti et al. 2005; Benigni and Battisti 1999; Hódar et al. 2003). After severe natural defoliation by *T. pityocampa* caterpillars, sugar and starch concentrations were found to be reduced by 54 and 74 %, respectively, in branch sapwood of adult *Pinus halepensis* trees in Samos, Greece, just prior to bud break. Only 5 weeks later, concentrations of sugars and starch recovered to about 65 % of undefoliated control trees (Körner, unpublished data). Hence, these mature trees were able to replenish their reserve pool, while at the same time, rebuilding their leaf canopy.

In the current study, we simulate various intensities of herbivory on *P. pinaster* trees just prior to the onset of the growing season, when natural processionary moth herbivory would result in maximum foliage loss. The impact on tree physiology was assessed by measuring NSC concentrations in different tree tissues and by measuring growth throughout the summer. Specifically, we addressed the following questions: (1) Does defoliation lead to a reduction in growth? (2) Is this accompanied by a reduction in mobile C reserves? (3) How are these responses affected by the intensity of defoliation? (4) If there is a reduction in C reserves in response to defoliation, how long does it take to rebuild those reserves? The primary goal of this work was to evaluate the potential of trees to cope with the damage inflicted by herbivory under concurrent summer drought, as the interactive effect of these stresses may exert rising impacts on the productivity of pine forests in the Mediterranean.

Materials and methods

Study sites and climate

The study was conducted during the growing season 2012 on young *P. pinaster* Ait. trees growing at a nature reserve (Riserva Naturale Statale di Montefalcone in Castelfranco di Sotto) near the city of Pisa, Italy. We defoliated and investigated trees at two forest sites (about 1 km apart) within the nature reserve, with young, small-statured *P. pinaster* trees growing at low stand density: Bosco Rastrello (43°45′N and 10°43′E, 93 m a.s.l.) and Bosco Tesoro (43°44′N and 10°44′E, 61 m a.s.l.). The silicaceous soil at both sites is acidic and nutrient-poor with a high proportion of clay in deeper layers (Cappelli et al. 2004). The pine stand in Bosco Rastrello was characterised by an open and relatively drier environment with shrubby vegetation dominated by *Erica arborea and E. scoparia, Ulex europaeus* and, *Cytisus scoparius*, with *Arbutus unedo* and *Rubus fruticosus* also present. The experimental trees in Bosco Tesoro were situated in a clearing within a more mesophilic mixed forest dominated by *Quercus cerris, Q. petraea* and *Carpinus betulus* and a ground cover dominated by herbaceous plants in spring.

Mean annual air temperature is 14.0 °C at the nearest weather station located 50 km away at 55 m elevation in Sesto Fiorentino, with mean January and July temperatures of 5.9 and 23.8 °C, respectively. Mean annual precipitation is 952 mm with little rain over summer (Fig. 1; Cappelli et al. 2004). Average to lower than average precipitation

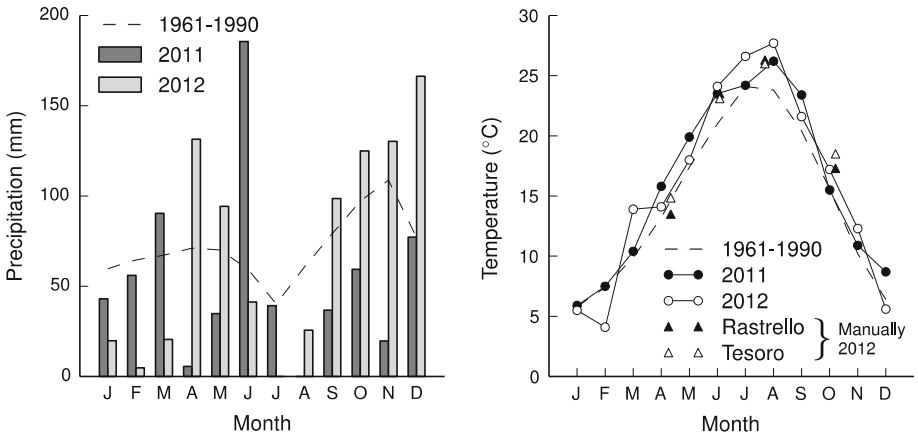

Fig. 1 Monthly mean precipitation and air temperature (1961–1990, *dotted line*) at the closest weather station in Sesto Fiorentino (FI, Italy, 55 m elevation, 50 km distant from the study sites; http://www.lamma.rete.toscana.it, 25 April 2013) compared to the treatment year (2012) and the previous year (2011). Additionally, manual soil temperature measurements during 2012 at the two study sites (*triangles*) are shown

occurred throughout 2011 (year before treatment), with the exception of massive rainfalls in June. In the 2012 (treatment year), precipitation was higher than average in April and May and from September to December, but much lower during summer (Fig. 1). Temperatures during most of the 2011 and 2012 growing seasons (April to August) were slightly above the long term mean (Fig. 1). Soil temperature was measured at both sites in the shade of the trees (two measurements per site) with a pocket thermometer GTH 175/PT (Conrad Electronics GmbH, Germany) at every sampling date. These measurements showed that the temperatures at both sites closely matched those measured at the closest weather station during 2012 and that there was no significant temperature difference between the two sites (Fig. 1).

Defoliation treatments

Twenty-four young, sun-exposed and healthy *P. pinaster* trees of similar age (6–12 years) and height (2.0–3.2 m) were selected at each site. The defoliation treatment took place before bud break, between 27 February and 2 March 2012 (i.e. during the peak period of caterpillar activity) and was performed randomly, switching at least once per day between the two sites during defoliation. Before defoliation, for each tree the number of year-cohorts of needles was counted, and the trunk diameters at 5 cm above ground and the tree heights were measured. At each site, 6 out of 24 trees were randomly assigned to one of the following treatments: 100 % defoliation (cutting all the needles of the trees, leaving only the non-green base of each needle fascicle), 50 % defoliation (randomly cutting half of the needles on each branch of a tree), complete defoliation of one large branch only, and no defoliation (controls). Buds were unaffected by the defoliation treatments and bud break, shoot elongation and new needle development occurred simultaneously in all defoliated and control trees in spring 2012.

Tissue sampling and biometric measurements

Samples approximately 5 cm long and 4 mm thick were collected from the 2011 growth from the distal part of a branch from each tree on five dates throughout the growing season in 2012: 27 February (prior to defoliation), 27 April (shoot elongation started), 19 June (needle elongation started), 7 August (needle and shoot elongation terminated), 23 October (end of the growing season). After cutting, previous season's needles, branch xylem and the secondary phloem including the active phloem and bark (manually removed from the xylem) were sampled separately from each branch. The samples were collected from south- and sun-exposed branches at breast height (middle part of the crown). On the last date (23 October), 5-cm-long samples of roots with a diameter of about 4 mm were sampled from each tree, the phloem was removed and the xylem samples were collected for NSC analyses. Also on the last sampling date, stem sapwood was cored at about 30 cm above ground, using a 0.5-mm stem corer (Haglöf, Sweden) and tree ring samples from 2009 to 2012 (age of the youngest tree at coring height) were collected from each tree for NSC analyses. Because of the young age of all sampled trees and the absence of heartwood, the stem sapwood samples of the latest 5-year rings are representative of the whole stem wood of the trees. All tissues were sampled between 10:00 a.m. and 4:00 p.m., and the sequence of sampling was randomised among treatments. Within 6 h of collecting, the sampled material was heated for 2 min in an oven at about 150 °C to denature enzymes (similar to the method described in Popp et al. 1996) and then dried at 80 °C for 4 h. After returning to the lab in Basel, Switzerland, the samples

were dried to weight constancy and ground into a fine powder in a mixer-mill (MM20, Retsch, Germany).

In April and August 2012, the lateral shoot length of three growing shoots (at breast height) and the respective shoot length of the previous year (2011) were measured on each tree, and the mean shoot length increment for 2011 and 2012, respectively, was calculated. For the trees with only a single branch defoliated, only the leading shoot of the defoliated branch was measured. In addition, in October 2012, for each tree a second stem core across the whole stem diameter was obtained a few centimetres above the first one for later dendrochronological analysis. These stem cores were mounted and glued firmly on a grooved wooden stick and air-dried. Then, the dried stem cores were sanded to allow clear tree ring readings on an electronic analysis bench (LINTAB, Rinntech and TSAP-Win 4.64 software).

NSC analysis

NSC was analysed according to Hoch et al. (2002). Briefly, ground plant material was suspended in distilled water and boiled for 30 min. The extract was then centrifuged, and an aliquot of the extract was treated with isomerase and invertase, to convert fructose and sucrose into glucose. Glucose was then enzymatically converted into gluconate-6-phosphate (hexokinase reaction, hexokinase from Sigma Diagnostics, St. Louis, MO, USA). Total glucose concentration (equivalent to the sum of the free sugars glucose, fructose and sucrose) was determined photometrically at 340 nm in a 96-well microplate photometer (HR 7000, Hamilton, Reno, NE, USA). The remaining water extract (including sugars and starch) was incubated over night with dialysed crude fungal amylase ("Clarase" from *Aspergillus oryzae*, Enzyme Solutions Pty Ltd., Croydon South, VIC, AUS) to break down starch to glucose. Total glucose (corresponding to NSC) was determined photometrically as described above. Starch was calculated by subtracting free sugars from NSC. The sugar and starch concentrations are expressed on a dry matter basis in mg g^{-1}.

Statistical analysis

Measurements of lateral shoot length were made on branches of different sizes and included leading and secondary branches. To standardise these measurements and ensure comparability among trees and sites, the lateral shoot length was expressed as a ratio of the treatment year's (2012) and the previous year's (2011) shoot length of the same branch. To account for age-dependent diameter effects, the stem basal area increment (BAI) was calculated from tree ring data for the years 2008–2012. During pre-treatment years, tree BAI was considerably lower in 2008 and 2009 compared to 2010 and 2011 (see also Fig. 3). This is probably an inverse age effect, since the trees were young (between 6 and 12 years old in 2012), still showing an exponential growth increase with age. The BAI of 2012 was divided by the mean BAI of 2010 and 2011 to standardise the increment in the treatment year (2012). This procedure accounts for a priori difference in vigour among the trees. Since the BAI of control trees and trees with only a single branch defoliated did not differ in 2012, BAI is not reported for trees with single branch defoliation.

Overall, tree growth was significantly higher at Bosco Tesoro than at Bosco Rastrello. However, we decided to combine both sites for statistical analyses for three reasons: (1) instead of absolute growth, we used relative growth standardised with pre-treatment growth for each tree, and there was no significant difference among sites for standardised growth values. (2) The trees' growth and NSC reactions to the defoliation treatments were identical between the two sites. (3) Finally, both sites were in close vicinity within the same forest. After testing data for normality and similarity of variances, significant differences between treatments in lateral shoot length ratios and BAI ratios were determined with a mixed model with defoliation as fixed effect. Sugar, starch and NSC concentrations for branch xylem, branch phloem and previous year needles ($n = 12$, except in two cases $n = 11$ due to lost samples) were analysed for significant differences between treatments using a mixed model with treatment and date as fixed effects. Sugars, starch and NSC in root and stem sapwood (sampled only on the last date) were analysed for significant differences analogously to the growth ratios ($n = 12$, except in one case $n = 10$ due to lost samples). Differences among treatments were tested for significance (at the $p < 0.05$ level) by Tukey–Kramer honestly significant difference (HSD) tests. All statistical analyses were performed using JMP Pro 11.0. (SAS Institute, Cary, NC, USA).

Results

Growth response

Generally, shoots grew less in 2012 compared to 2011 (shoot length ratios of 2012/2011 below 1 for all treated and control trees; Fig. 2), which was probably due to the drier summer condition in 2012 compared to 2011 (Fig. 1). The shoot length increment in Bosco Tesoro was significantly higher than in Bosco Rastrello if absolute values were considered (mean ± SD of 32.4 ± 7.5 and 13.4 ± 8.6 cm, respectively, for control trees), but the standardised values (ratios of 2012/2011) were similar and not significantly different between sites for all treatments. While there was no significant difference in lateral shoot

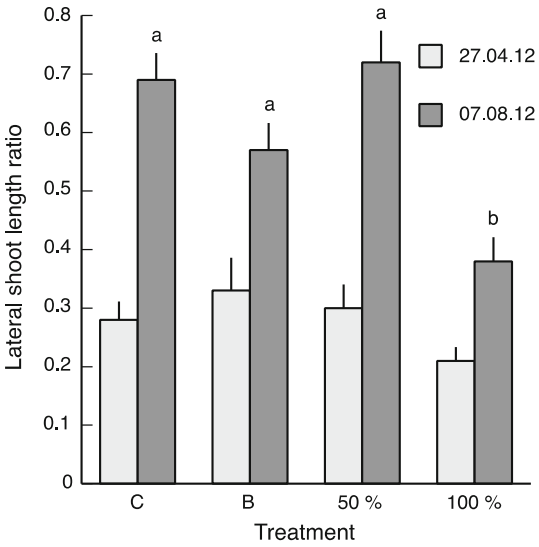

Fig. 2 Lateral shoot length ratio (shoot increment 2012 relative to shoot increment 2011) for the different treatment groups. C, controls; B, single branch defoliation; 50 %, 50 % defoliation; 100 %, complete defoliation. Values are mean + SE ($n = 12$). *Different letters* significant differences between treatments in August ($p < 0.05$, Tukey–Kramer HSD test)

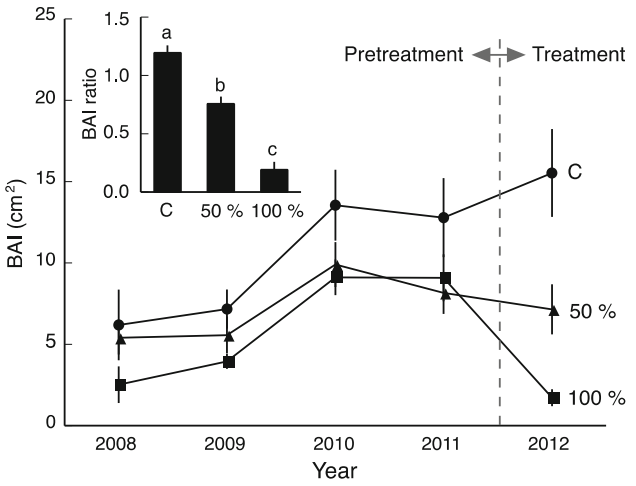

Fig. 3 Growth response of pines (basal area increment, BAI) for the previous 4 years plus the treatment year (mean ± SE of 11–12 trees per treatment, absolute values). *Inset diagram* BAI ratio [2012/0.5 (2010 + 2011)] for the different treatment groups (mean + SE, $n = 11$–12 trees). *Different letters* significant differences between treatments ($p < 0.05$, Tukey–Kramer HSD test). C, controls; 50 %, 50 % defoliation; 100 %, complete defoliation

length ratios between treatments at the end of April (about 1–2 weeks after bud break, $F = 1.6$, $p = 0.209$), shoot growth was significantly affected by defoliation in August ($F = 9.6$, $p < 0.001$; Fig. 2). Defoliation led to a significant shoot length decrease in 100 % defoliated trees compared to controls (−45 %), but neither 50 % defoliation nor single branch defoliation had a significant effect on new shoot growth (+4 % and −17 %, respectively).

Similar to shoot growth, BAI values of controls were higher in Bosco Tesoro than in Bosco Rastrello (data not shown), but again, the standardised values (BAI ratios) were similar between sites. Control trees tended to have higher absolute BAI values already prior to the defoliation year (2008–2011, Fig. 3). However, even if this a priori difference is taken into account by standardising the 2012 BAI with the mean BAI in 2010 and 2011 (BAI ratio), there was a highly significant effect of defoliation on BAI ($F = 59.5$; $p < 0.001$). The reduction in stem increment in 2012 compared to control trees was strong and significant for 100 % defoliated trees (−84 %) as well as for 50 % defoliated trees (−37 %, Fig. 3).

NSC response

Reserve carbohydrate concentrations varied across the season in all analysed tissues (Fig. 4). In control trees, sugars in branch xylem peaked in February, decreased during spring growth until June, and increased for the rest of the season until October. Starch concentrations in branch xylem were low in February, peaked in April (prior to bud break), decreased until August and remained constant until October. Total NSC concentrations in branch xylem remained high until April (the decrease in sugars was compensated by the increase in starch), decreased until August and then increased slightly until the end of the growing season. In branch phloem, carbohydrate concentrations were always higher than in xylem, but showed a similar seasonal course. Sugars did not change as much over the season in branch phloem as in branch xylem, and hence, total NSC concentrations in branch phloem peaked in April, since starch increases were higher than decreases in sugars. Previous year needles showed similar sugar concentrations as branch phloem, whereas starch concentrations were higher than in branch phloem. Sugar concentrations in needles were constant throughout the growing season, while starch and total NSC concentrations were high in April, decreased until August and then remained constant until October (there is no needle data prior to the defoliation).

The defoliation treatments had significant effects on carbohydrate concentrations in branch xylem, branch phloem and previous year needles for all compounds, except for sugars in branch xylem (Table 1). There were also significant time × treatment interactions in these tissues for all compounds, with the exception of sugars in needles. Generally, treatment effects were more pronounced for starch than for sugars (Fig. 4), but the effects found in the first half of the season were not sustained in most cases. By the end of the growing season, NSC concentrations were

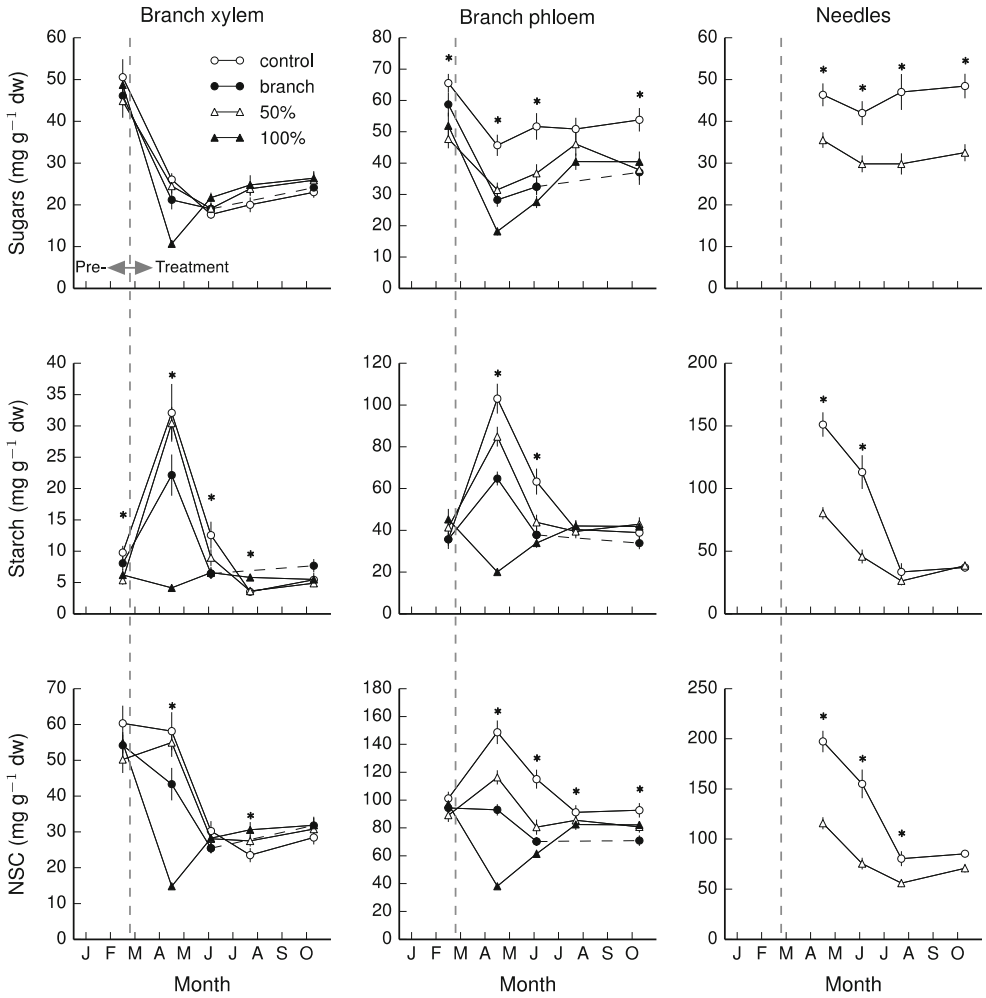

Fig. 4 Seasonal variation of sugar, starch and total NSC concentrations (mean ± SE, $n = 11$–12 trees) for branch xylem, branch phloem and previous year needles separated into the four treatments. *Asterisks* indicate significant differences between treatments ($p < 0.05$, Tukey–Kramer HSD test). Note the different *y*-axis scales. For obvious reasons, we show no foliage data for trees with only a single branch defoliated and for 100 % defoliated trees. Since trees with only a single branch defoliated were not sampled in August, the trend between June and October is represented with *dashed lines*

Table 1 Mixed model for the influence of defoliation on carbohydrate reserves in branch xylem, branch phloem and 1-year-old needles (see "Materials and methods" for details)

	Branch xylem		Branch phloem		Needles	
	F	*p*	*F*	*p*	*F*	*p*
Sugars						
Date	145	<0.001	45.2	<0.001	3.0	0.050
Treatment	0.9	0.424	29.9	<0.001	16.4	<0.001
Date × treatm.	3.8	<0.001	2.0	0.042	0.3	0.867
Starch						
Date	68.0	<0.001	44.1	<0.001	120	<0.001
Treatment	18.7	<0.001	28.3	<0.001	36.3	<0.001
Date × treatm.	11.5	<0.001	19.5	<0.001	10.5	<0.001
NSC						
Date	62.9	<0.001	14.6	<0.001	89.1	<0.001
Treatment	10.4	<0.001	62.7	<0.001	45.2	<0.001
Date × treatm.	10.9	<0.001	20.6	<0.001	7.0	<0.001

again similar among all treatments, except for sugars in previous year needles and branch phloem (Fig. 4).

In branch xylem, sugar concentrations did not differ between treatments at any time. However, starch concentrations of 100 % defoliated trees were significantly reduced in April (2 months after defoliation) compared to controls (the peak during April seen in control trees is completely missing, Fig. 4). In response to 50 % and single branch defoliation, starch concentrations in April were also reduced but less than after 100 % defoliation (the April peak is still evident). The higher starch concentrations in branch xylem during spring in the single defoliated branches compared to completely defoliated trees indicated that C reserves were imported into the defoliated branches from the surrounding undefoliated branches. By the end of the season, starch concentrations in branch xylem were similar for all treatments. In branch phloem, sugar concentrations were lower for all defoliation treatments (100,

Table 2 Mixed model for the effects of partial and complete defoliation on reserve carbohydrates in stem and root sapwood in October (see "Materials and methods" for details)

	Stem sapwood		Root sapwood	
	F	*p*	*F*	*p*
Sugars	1.2	0.312	0.2	0.977
Starch	0.4	0.775	0.3	0.756
NSC	0.9	0.450	0.1	0.866

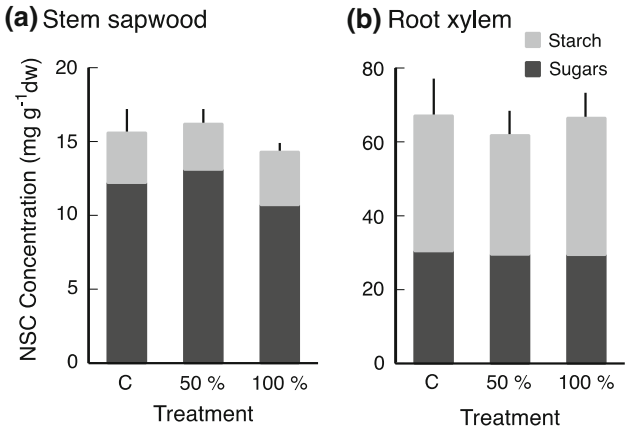

Fig. 5 NSC concentrations (mean + SE, $n = 11$–12 trees) on 23 October 2012 for stem sapwood (**a**) and root xylem (**b**) separated into the different treatments (C, controls; 50 %, 50 % defoliation; 100 %, complete defoliation). There are no significant differences between treatments (at $p < 0.05$, Tukey–Kramer HSD test). Note the different *y*-axis scales

50 % and single branch defoliation) during the whole season compared to controls. However, because this was the case already prior to defoliation (Fig. 4), the lower sugar concentrations might be unrelated to the treatments. Starch concentrations in branch phloem showed a similar response to the treatment as in branch xylem. In previous year needles, sugar concentrations remained constant throughout the season (April to October) for 50 % defoliated trees, but they showed significantly reduced concentrations compared to control trees (Fig. 4). Similar to branch phloem and xylem, starch concentrations in needles showed a reduced April peak in 50 % defoliated trees, but again, by the end of the growing season, this effect had vanished. In stem sapwood and root xylem, sugar and starch concentrations did not differ significantly between treatments at the end of the growing season (Table 2; Fig. 5).

Discussion

This in situ defoliation experiment under Mediterranean growth conditions revealed that the loss of previous season needles before bud break led to severe reductions of both annual shoot extension and BAI in 100 % defoliated trees and to a lesser extent in 50 % defoliated trees. While defoliation led to a significant decline of carbohydrates in branch xylem and phloem during the first half of the growing season (with the magnitude of reduction mirroring the defoliation intensity), concentrations were back to the level of control trees by the end of the growing season in October.

Severe growth reductions have been observed previously during natural processionary moth attacks. For example, Jacquet et al. (2013) found that following a severe outbreak of processionary moths in South-western France, radial stem growth in mature *P. pinaster* trees was significantly reduced for at least 2 years after defoliation. In their review, Jacquet et al. (2012) summarised the results of previous studies and showed that natural and artificial defoliation resulted in significant reductions of tree growth with increasingly negative growth effects the higher the defoliation rates. These authors further suggested that the effect levels out at about 50 % growth reduction, and that the overall growth decline after defoliation was stronger in young trees. Accordingly, the 6–12-year-old trees investigated in our study also showed a very strong growth decline of about −65 % in completely defoliated trees. Following an experimentally induced 100 % defoliation by the processionary moth for eleven consecutive years (by transferring caterpillar nests on trees), Palacio et al. (2012) reported growth reductions of even up to 91 %. In any case, our study corroborates previous observations of a strong negative effect of early season defoliation on the growth of pines in the same year.

The seasonal dynamics and magnitude of carbohydrate concentrations in undefoliated *P. pinaster* were similar to findings of previous studies for *Pinus* species (Ericsson et al. 1985; Oleksyn et al. 2000; Hoch et al. 2003). Generally, sugar concentrations remained fairly constant throughout the growing season. The starch peak in branch wood and needles prior to bud break is characteristic for evergreen conifers (Fischer and Höll 1991, 1992; Hoch et al. 2003; Körner 2003) reflecting the high availability of C, due to active photosynthesis in early spring while meristems are still inactive (no C demand for shoot and stem growth). Sugars were much less affected by the defoliation compared to starch, likely because low molecular sugars feature important cell functions, such as osmoregulation or as metabolites. Starch, in contrast, is an osmotically inactive macromolecule, which is used as a C storage compound without additional cell physiological functions.

Early growth of new shoots depends largely on current-year photosynthates (Hansen and Beck 1990, 1994). Although needles were completely missing in 100 % defoliated trees (at least until the new shoots started

growing), carbohydrate concentrations in branches fell only slightly below initial (February) values and considerable mobile C pools were maintained throughout the growing season. This could indicate either no or only a very transient C source limitation for shoot growth, which confirms results from Palacio et al. (2012) in evergreen *P. nigra* trees and from Kosola et al. (2001) in deciduous hybrid poplar. But, since growth of 100 % defoliated trees was significantly reduced, this would also concur with the hypothesis that C reserves were kept at a certain level at the cost of growth, hence occurring while growth is still C limited (Wiley et al. 2014). However, assuming a rapid C autonomy of emerging foliage in evergreen conifers, similar to developing leaves in deciduous broadleaved trees (Landhäusser 2011), there might be overall little demand on reserves for re-growth after defoliation. In any case, keeping up a certain safety pool of C reserves may be necessary for trees to mitigate further attacks (Chapin et al. 1990). Many insect pests have population dynamics with periodical outbreaks, with consecutive years of high impact. Exhausting reserves in 1 year would therefore raise vulnerability.

Reduced growth after defoliation accompanied by high NSC levels may also be explained by several other causes:

1. Parts of the storage C pool of a tree may not be freely available (sensu sequestered; Millard and Grelet 2010). However, experiments that severely reduced C uptake without defoliation by either exposure to extreme low atmospheric CO_2 concentrations (e.g. Schädel et al. 2010; Zhao et al. 2013) or by strong shading (e.g. Sevanto et al. 2014) caused a very strong reduction of starch in the middle of the growing season, showing that these reserves should be readily available if needed.
2. Since defoliation not only removes photosynthetic capacity, but also represents a major drain of important nutrients such as N and P, periodic nutrient limitation may be another reason for reduced growth (Millard et al. 2001). This is especially true in evergreen trees, which store most of their nutrients in needles. Reduced growth in defoliated trees might therefore be driven by the abrupt shortage of nutrient supply. The loss of nutrient reserves that were stored in old needles might also induce higher investments in root growth and fine root foraging, at the cost of aboveground growth. However, in a 3-year experiment on defoliated poplar trees, Kosola et al. (2001) did not find changes in root production following defoliation and a significantly depressed uptake of nitrate and ammonium, contradicting this hypothesis. In addition, even a preferential shift of new biomass to foliage at the cost of reduced root growth after defoliation has been described in seedlings of *Pinus resinosa* (Reich et al. 1993) and saplings of *Eucalyptus globulus* (Eyles et al. 2009).
3. The observed decline in stem basal area at an otherwise smaller (100 % defoliation) or missing (50 % defoliation) branch length response in defoliated pines may simply reflect a reduced demand for conduits due to the reduced needle canopy (allometric balance). Such a balance can be triggered by defoliation through hormonal signals. Gibberellins are involved in the direct control of cambial and length growth in conifers and a major part of a conifer's gibberellins are found in needle fascicles (Wang et al. 1997). A positive correlation between gibberellins and growth can at least partly explain the reduced BAI and shoot length after defoliation (loss of gibberellin signals).

The fast recovery of NSC concentrations suggests that photoassimilation in new foliage rapidly became a C source for storage, presumably enhanced by an up-regulation of the photosynthetic capacity of needles (Reich et al. 1993; Huttunen et al. 2007; Palacio et al. 2012). Palacio et al. (2012) showed that despite the loss of nitrogen by herbivory, the nitrogen concentrations in remaining needles increased in *P. nigra* trees in response to processionary moth defoliation, suggesting enhanced photosynthesis in remaining foliage which may have compensated for the partial loss of foliage immediately after defoliation. This could also explain why our moderate defoliation treatment (50 %) had minimal affect on NSC in branch phloem and xylem and on shoot growth. However, previous year needles of 50 % defoliated trees lost 47 % of their NSC reserves and these trees had a 37 % loss of BAI compared to controls. It has been reported that older needles export parts of their current assimilates immediately after bud burst with enhanced rates of this transfer during summer, when the sink demand of the new shoots has ceased (Hansen and Beck 1994). If a large proportion of stored assimilates is consumed for shoot growth (the reduced April peak in needle starch concentrations in 50 % defoliated trees compared to controls), the translocation of assimilates into the stem might have diminished or the demand for C was lower given the reduced conduit requirement. This could explain the reduction in BAI and NSC in needles while shoot length remained unaffected in 50 % defoliated trees.

When only single branches were defoliated, these branches also had lower carbohydrate concentrations in branch xylem and phloem compared to controls, but the reduction was far less pronounced as in branches of 100 % defoliated trees. Such individually defoliated branches appear to receive C compounds from the rest of the tree. It

has been shown that, when photosynthetic tissues is removed or greatly reduced, photosynthates can be imported into the growing tissues from other parts of the tree (e.g. Obeso 1998; Hoch 2005), although under "normal" conditions branches are relatively C autonomous during the growing season (for a review see Sprugel et al. 1991).

In contrast to other studies, we found no differences in carbohydrate concentrations in response to defoliation in root and stem wood at the end of the season (October). Li et al. (2002) reported a reduction of NSC in root and stem wood of 100 % defoliated small *P. cembra* saplings in situ at the Swiss treeline by 72 and 47 %, respectively, by the end of the short growing season. Vanderklein and Reich (1999) show that 50 % defoliated field grown seedlings of *P. resinosa* had a 51 % reduction of starch in roots but no reduction in stems by the end of the season. The different effects of defoliation on root carbohydrate concentrations in these studies might relate to the special local setting (e.g. treeline) or the use of seedlings rather than larger trees. Under normal conditions, carbohydrate concentrations in stem sapwood of pine trees are fairly constant over the growing season (Fischer and Höll 1992; Hoch et al. 2003). It seems that in the case of a need, carbohydrate reserves are first mobilised from tissues other than stems.

In conclusion, our study revealed that simple C source–sink relationships are insufficient to explain growth reductions in response to defoliation. The data presented here indicate a surprisingly small effect of 50 % defoliation on NSC pools and a rapid re-establishment of NSC pools even after 100 % defoliation. It was not possible to unambiguously separate the various possible causes for reduced growth after defoliation at otherwise high NSC levels (priority of C storage, C sequestration, nutrient limitation, hormonal signals, or allometric balance). Yet, whatever the cause, it is remarkable that NSC reached control levels by the end of the dry and hot Mediterranean summer in 100 % defoliated trees after the new needle cohort was established. NSC and especially starch were never depleted, and hence, C starvation (i.e. complete consumption of C reserves) does not seem to occur even after severe defoliation. Our data indicate some additional C demand from reserves during the most active re-growth in defoliated trees, but towards the end of the season, when all shoot growth and most needle growth was completed, NSC concentrations rapidly rose to pre-treatment levels. However, it remains unresolved if a shift in C allocation priority from structural growth to storage (Wiley and Helliker 2012; Wiley et al. 2014) has taken place.

Author contribution statement GH and CK designed and supervised the experiment. EP performed the experiment. EP and GH analysed the data. EP and GH wrote the manuscript. CK provided scientific and editorial advice for the text.

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Conflict of interest The authors declare that they have no conflict of interest.

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