Combined effects of defoliation and water stress on pine growth and non-structural carbohydrates

Jean-Sébastien Jacquet\textsuperscript{1,2,6}, Alexandre Bosc\textsuperscript{3,4}, Anthony O’Grady\textsuperscript{5} and Hervé Jactel\textsuperscript{1}

\textsuperscript{1}INRA, UMR1202 BIOGECO, F-33612 Cestas, France; \textsuperscript{2}Univ. Bordeaux, UMR 1202, BIOGECO, F-33405 Talence, France; \textsuperscript{3}INRA, UMR 1391 ISPA, F-33140 Villenave d’Ornon, France; \textsuperscript{4}Bordeaux Sciences Agro, UMR 1391 ISPA, F-33170 Gradignan, France; \textsuperscript{5}CSIRO Sustainable Ecosystems, Private Bag 12, Hobart, Tasmania 7001, Australia; \textsuperscript{6}Corresponding author (jacquet@pierroton.inra.fr)

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Climate change is expected to increase both pest insect damage and the occurrence of severe drought. There is therefore a need to better understand the combined effects of biotic and abiotic damage on tree growth in order to predict the multi-factorial effect of climate change on forest ecosystem productivity. Indeed, the effect of stress interactions on tree growth is an increasingly important topic that greatly lacks experiments and data, and it is unlikely that the impact of combined stresses can be extrapolated from the outcomes of studies that focused on a single stress. We developed an original manipulative study under real field conditions where we applied artificial defoliation and induced water stress on 10-year-old (~10 m high) maritime pine trees (\textit{Pinus pinaster} Ait.). Tree response to combined stresses was quantitatively assessed following tree secondary growth and carbohydrate pools. Such a design allowed us to address the crucial question of combined stresses on trees under stand conditions, sharing soil supplies with neighboring trees. Our initial hypotheses were that (i) moderate defoliation can limit the impact of water stress on tree growth through reduced transpiration demand by a tree canopy partly defoliated and that (ii) defoliation results in reduced non-structural carbohydrate (NSC) pools, affecting tree tolerance to drought. Our results showed additive effects of defoliation and water stress on tree growth and contradict our initial hypothesis. Indeed, under stand conditions, we found that partial defoliation does not limit the impact of water stress through reduced transpiration. Our study also highlighted that, even if NSC in all organs were affected by defoliation, tree response to water stress was not triggered. We found that stem NSC were maintained or increased during the entire growing season, supporting literature-based hypotheses such as an active maintenance of the hydraulic system or another limiting resource for tree growth under defoliation. We also observed a significant decrease in root carbohydrates, which suggests a shift in the root carbon balance under defoliation. The decrease in carbohydrate supply under defoliation may not counterbalance the carbon use for mineral and water uptakes or a translocation to other tissues.

**Keywords:** drought, forest productivity, herbivory, multiple stresses, radial growth.

**Introduction**

The effects of single stresses including abiotic stresses like drought (Breda et al. 2006) and biotic stresses such as insect defoliation (Pinkard et al. 2011) on tree growth have been well documented. However, they are likely to occur concomitantly in the real world, and increasingly often in the future as climate change will increase their frequency. At a global scale, it has been reported that heat waves are likely to be more intense, more frequent and longer lasting in a future warmer climate (Coumou and Robinson 2013). They may result in increased soil and plant evapotranspiration, leading to drought. Moreover, longer periods between rainfall events and a tendency for lower
precipitation during summer are expected in some regions (Solomon et al. 2007), potentially causing drought. In southwestern France, average temperature is expected to increase by +2.7 °C and average rainfall to decrease between −182 and −219 mm between the recent past (1970–99) and the distant future (2070–99) (Brisson and Levrault 2010) according to the A2 scenario (Nakicenovic et al. 2000) and the CNRM-CM3 GCM model (Salas et al. 2005). This results in a higher risk of water stress in summer (Brisson and Levrault 2010). At the same time, climate change is expected to increase insect damage (Logan et al. 2003, Netherer and Schof 2010). As an example, pine processionary moth (PPM) Thaumetopoea pityocampa (Dennis & Schiff) is the main insect defoliator of pine and cedar species in southern Europe (Devkota and Schmidt 1990; Masutti and Battisti 1990) and its range has spread toward higher latitudes and altitudes in the last decade, probably in response to rising winter temperatures (Battisti et al. 2005, Robinet et al. 2007). There is therefore a need to better understand the combined risks of biotic and abiotic stresses on tree growth to provide a more holistic view of the effect of climate change on forest productivity.

To date, contrasting hypotheses have been suggested dealing with that issue. The compensatory continuum hypothesis predicts a lower tolerance to damage for plants under reduced resource availability (Maschinski and Whitham 1989). A recent meta-analysis has demonstrated that damage by insect defoliators is significantly higher under water-stressed conditions (Jacquet et al. 2011). Furthermore, it has also been reported that defoliation may limit plant tolerance to challenging environmental conditions (Valladares et al. 2007). However, other studies have suggested the opposite combined effect, with woody plants more tolerant to defoliation when resources are limiting (Hawkes and Sullivan 2001, Wise and Abrahamson 2007). Thus, it is not clear whether the effects of several interacting stresses would be additive, synergistic or even antagonistic.

Defoliation itself may have significant detrimental effects on tree growth (Chen et al. 2002) via a decreased photosynthetic leaf area and thus reduced carbohydrate supply (Eyles et al. 2009, Pinkard et al. 2011, Jacquet et al. 2012). Indeed, it has been suggested that non-structural carbohydrates (NSC), usually considered as a carbon reserve for energy and biosynthesis, mediate tree growth responses to defoliation (Eyles et al. 2009, Pinkard et al. 2011). In many cases, moderate and severe defoliation resulted in reduced NSC concentrations (Ericsson et al. 1980, Bauer et al. 2000, Li et al. 2002, Hudgeons et al. 2007, Palacio et al. 2011, Quentin et al. 2011, Jacquet et al. 2013, but not always in case of light defoliation, e.g., Van der Heyden and Stock 1995), which might in turn reduce carbon allocation to tree growth (Trumble et al. 1993).

Similarly, drought can also cause declines in growth via its impacts on cell turgor and carbon assimilation resulting from stomatal conductance regulation (Bréda et al. 2006, McDowell et al. 2008). However, it is unclear how these ecophysiological mechanisms interact in a combination of water and defoliation stress (Gieger and Thomas 2002, Quentin et al. 2012). By reducing foliar biomass, insect defoliation may reduce the transpirational demand from the canopy and thus reduce the detrimental effects of drought on tree metabolism (Quentin et al. 2012). The loss in carbohydrate production due to reduced photosynthesis in defoliated trees may limit the carbohydrate supply to the root system, thus limiting its capacity to increase root production and exploit available soil water under drought conditions (Gieger and Thomas 2002). Furthermore, as NSC have been mentioned as crucial to maintaining hydraulic functioning in trees (Sala et al. 2012), their depletion under defoliation may trigger tree resistance to water stress.

While all of these processes have been proposed, to date there have been very few experimental studies examining both defoliation intensity and water stress to determine their combined effects on tree growth (but see McGraw et al. 1990, Kolb et al. 1999, Quentin et al. 2012). To address this gap, we developed a manipulative study under field conditions, where we combined artificial defoliation and water stress in plots of 10-year-old (~10 m high) maritime pine trees (Pinus pinaster Ait.). We monitored both radial tree growth dynamics and carbohydrate pools in all tree organs in order to assess tree response to combined stresses. More particularly, we tested the following two hypotheses:

(i) Moderate defoliation can limit the impacts of water stress on pine tree growth through reduced transpirational demand by a tree canopy partly defoliated, i.e., antagonistic effects of water stress and defoliation stress.

(ii) Defoliation results in reduced NSC pools, affecting tree tolerance to drought (maintenance of the hydraulic system and expansion of the root system).

Materials and methods

Site

The field experiment was conducted in a 10-year-old, monospecific maritime pine (P. pinaster) plantation at the INRA Forest Research Centre at Cestas, south-western France (latitude: 44°44′35.822″N, longitude: 0°46′58.914″W). The stand density was 1250 trees per ha. The climate of the region is thermo-Atlantic (mean annual temperature of 13 °C, mean annual precipitation of 977 mm) with wet winters and marked drought in late summer (August–September). During the study year (2011), precipitation was 638 mm and monthly average maximum and minimum temperatures were 27.7 and 1.3 °C, respectively. The region is flat with podzol soils established on several meters of sandy deposit. The forest in the Landes area is productive thanks to maritime pine’s tolerance to acidic soils and low mineral nutrition (Trichet et al. 2009).
Treatments and experimental design

The effects of defoliation and water supply on tree growth were tested in a complete factorial design with four replications. Within the pine stand, we selected eight plots of three neighboring trees of similar height and diameter (for a total of 24 trees). Four plots were allocated to the well-watered treatment and four to the drought treatment. Within each plot, one tree was 100% defoliated, one was 50% defoliated and the last tree was left intact (control). Both defoliation and water treatments were applied at the same time, in February 2011.

Well-watered plots were irrigated with sprinklers operating 15 min h⁻¹ from 9 pm to 6 am to provide a rainfall equivalent of 9 mm m⁻² day⁻¹ (from 1 June 2011 to 30 November 2011). The drought treatment was established using rainfall exclusion shelters placed around tree trunks under the canopy at 1 m above ground level. Our shelters were designed for full rainfall exclusion, which means that the percentage of annual rainfall was close to zero for this treatment. The water was collected in gutters and conducted 10 m outside the plot in drainpipes. In addition, to reinforce the drought treatment, neighboring trees (Appendix 1 available as Supplementary Data at Tree Physiology Online) were forced to use the water supply within the plot by cutting their external roots. Within each plot, the potential exploration area for the roots was diminished while the leaf area was unchanged. The leaf area index was decreased by 50% to intensify water stress. Predawn leaf water potential (Ψpd) was measured on one needle (1 year old) collected in the upper canopy, using a pressure chamber and recorded three times during the experiment (12 July, 9 August and 17 October 2011) to assess the progression of water stress.

Artificial defoliation was carried out to mimic PPM defoliations (Jacquet et al. 2012) in February 2011 which coincided with the end of the natural PPM feeding period. Pine processionary moth caterpillars feed on older needles and shift to current-year needles when the former are entirely consumed. Old foliage contributes ~50% of tree crown area in maritime pine (Porté et al. 2000). Trees were then estimated to be 50% defoliated if all of the mature needles were cut and 100% defoliated if all the mature and current-year needles were removed. Needles were snipped manually with scissors up to 0.5 cm from the brachyblast in order to mimic PPM feeding behavior. Removed needle material was left at the tree base (see Jacquet et al. 2013). As similar radial growth responses were found to natural and artificial defoliation in maritime pine stands of similar age (10 years old) (Jacquet et al. 2013), we were confident in using artificial defoliation as a surrogate of natural PPM defoliation in this study.

Radial growth measures

The circumference at breast height (1.30 m above ground level) of each sampled tree was measured weekly from February 2011 to November 2011 using mechanical dendrometers (0.1 mm) in order to capture intra-annual growth dynamics.

Prior to defoliation, there were no significant differences between the mean circumferences of undefoliated and defoliated trees (28.5 cm for 0% defoliation, 26.5 cm for 50% defoliation, 28.09 cm for 100% defoliation, F = 0.04, P > 0.05). For each individual tree, we calculated a normalized circumference increment (cm) to allow for growth comparisons between trees (Eq. (1)).

$$C(d) = (C(d) - C(d_0)) \times \frac{C(d_0)}{C(d_0)}.$$

where C(d0) is the initial circumference of the tree on 1 February 2011, C(d) the mean initial circumference of all sampled trees and C(d) the circumference of the tree at date d. Annual radial growth was then C(d(end)) with C(d(end)) being the tree circumference on 30 November 2011.

Carbohydrate concentrations

The concentrations of NSC in needles, roots, stem phloem and stem sapwood of all 24 sampled trees were estimated in November 2011, i.e., at the end of the growing season. Stem NSC (sapwood and phloem) were also sampled in May and July 2011 to characterize carbohydrate dynamics in the stem. For these 24 trees, stem tissues (phloem and sapwood) were collected at 30 cm above ground level. Phloem tissue was sampled first using a 16-mm-diameter corer after removing the bark. Then, sapwood shavings were extracted using a drill bit (3.4 cm deep, 1.1 cm diameter). One main root was extracted until its diameter reached 5 mm. Five needles from all age classes were sampled from a main branch. For roots and needles, all tissues were ground together. Samples were frozen at −80 °C at the time of sampling using liquid nitrogen, before lyophilization.

Extraction of sugars was performed on ~30 mg of tissue powder following a method derived by Moing et al. (1992) and modified according to the following purification procedure. The extracts were purified on polyvinyl polypropyridine (PVPP, Sigma-Aldrich, Lyon, France) for eliminating polyphenols and on anion exchangers (AG1*8 100–200 Mesh, Bio-Rad Life Science, France, HCO₃⁻ form) and activated carbon (Darco® powder, 100 mesh particle size, Sigma-Aldrich, Lyon, France). Extracts were desiccated and then dissolved in ultrapure water for high-performance liquid chromatography analyses. Quantification of glucose, fructose and sucrose concentrations was done at 85 °C with a flow rate of 1 ml min⁻¹ of ultrapure water, using a Gilson 715 system (Gilson International France, Roissy, France) equipped with a column Aminex FAST CARBOHYDRATE and a precolumn Micro-Guard Carbo-C Refill Cartridge 30 × 4.6 mm (BIO-RAD, Marnes-La-Coquette, France). The detection was done by refractometry (RI2000, DURATEC Analysentechnik GmbH, Hockenheim, Germany). Soluble sugar (SS) concentrations were calculated as the sum of glucose, fructose and...
sucrose concentrations. From the pellet obtained after SS extraction, starch content was quantified as glucose equivalent after hydrolysis with amyloglucosidase (Boehringer 1984) at an absorbance of 340 nm on a microplate scanning spectrophotometer (Power wave 200, Biotek Instruments, Seralbo Technologie, Bonneuil sur Marne, France).

To allow consideration of the relative contribution of the different organs to the tree carbohydrate storage, organ carbohydrate pool sizes (in g tree$^{-1}$) were calculated as the product of carbohydrate concentration measurements and estimated organ biomasses. For each tree, individual organ biomasses (roots, needles, stem phloem and stem sapwood) were calculated using allometric equations on $P.\ pinaster$ based on trunk circumference and tree age (Porté et al. 2002). Using those allometric equations, the variation of biomass across treatments within one growing season was considered negligible relative to the importance of organ pool sizes in 10-year-old maritime pine trees. Organ biomasses were then only used as an integration factor. Indeed, a potential small variation in stem and root biomass would be negligible on 10-year-old maritime pine trees compared with initial biomasses. Furthermore, if artificial defoliation (applied in February 2011) has an obvious direct effect on 2008–2010 needle biomass, Jacquet et al. (2013) demonstrated that defoliation has no effect on the newly produced biomass (following defoliation) of 2011 needles. The sum of SS and starch measured was referred to as total NSC (TNC). As initial tree sizes could slightly differ between trees and influence carbohydrate pool size (through differences in total biomass), we took as a normalized factor for initial tree size while calculating the mean values per treatment the ratio between the average total biomass of all sampled trees and the total biomass of the sampled tree.

Whole-tree SS, starch and TNC pool sizes (g tree$^{-1}$) were calculated as the sum of contents in the different compartments.

**Statistical analyses**

Using generalized linear model comparison, we found that the plot effect (as a random factor) was never significant ($P > 0.05$). Then, we performed simple analyses of variance (ANOVA) to test the effect of water and defoliation treatments (as fixed factors) and their interaction on radial tree growth (CI) and carbohydrate pool sizes (g tree$^{-1}$). All analyses were performed using R (AOV package, Chambers et al. 1992). Post hoc comparisons of means were performed using Tukey’s tests to determine significant differences among treatments. For each model, residuals were verified by graphical analyses to assess the assumptions of normality and homoscedasticity.

**Results**

**Predawn leaf water potential only affected by the water treatment**

The water treatment had a significant effect on predawn leaf water potential (Table 1). As expected, it was significantly lower in the water stress treatment than in the irrigated treatment at the three dates of assessment ($−0.37 ± 0.029$ vs $−0.83 ± 0.14$ MPa in the irrigated and stressed plots, respectively, in August 2011). Defoliation had no significant direct or interactive effects on leaf water potential, suggesting that the three trees of each plot did share the same water resources.

**Annual growth affected by both defoliation and water availability**

Both defoliation rate and water treatments significantly affected annual stem growth with no significant interaction between the two factors (Table 2).

In the well-watered plots, the annual radial growth was significantly reduced by 88% in 100% defoliated trees compared

**Table 1. Summary of two-way ANOVA results showing the effects of water stress, defoliation and their interaction on predawn water potential.**

<table>
<thead>
<tr>
<th>Predawn water potential</th>
<th>Factor</th>
<th>df</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 December 2011</td>
<td>Defoliation</td>
<td>2</td>
<td>0.69</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Water treatment</td>
<td>1</td>
<td>81.39</td>
<td>1.736 × 10$^{-8}$</td>
</tr>
<tr>
<td></td>
<td>Defoliation × water treatment</td>
<td>2</td>
<td>0.39</td>
<td>0.68</td>
</tr>
<tr>
<td>8 September 2011</td>
<td>Defoliation</td>
<td>2</td>
<td>3.95</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td>Water treatment</td>
<td>1</td>
<td>50.78</td>
<td>6.64 × 10$^{-7}$</td>
</tr>
<tr>
<td></td>
<td>Defoliation × water supply</td>
<td>2</td>
<td>4.07</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>Defoliation × water treatment</td>
<td>2</td>
<td>1.18</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Water treatment</td>
<td>1</td>
<td>8.14</td>
<td>0.0098**</td>
</tr>
</tbody>
</table>

$F$-value statistics were calculated with ANOVA at the tree level. **$P < 0.01$, ***$P < 0.001$.**
with control undefoliated trees, but not in 50% defoliated trees (Figure 1).

Independent of their defoliation level, water-stressed trees showed significantly reduced annual radial growth (Table 2, Figure 1). The relative growth loss in water-stressed trees compared with non-stressed trees remained constant across the defoliation classes (−35 ± 26, −32 ± 23, −22 ± 77% of relative reduction for 0, 50 and 100% defoliation, respectively).

**Growth intra-annual dynamics**

We observed (Figure 2) a clear decoupling of growth in 100% defoliated trees when compared with the 50% and control trees from the beginning of the growing season (March 2011). Differences in growth dynamics between water-stressed and well-watered trees appeared in April for undefoliated trees, in May for 50% defoliated trees and in July for 100% defoliated trees, suggesting that defoliation intensity delayed the onset of water stress.

*Carbohydrate pools at the end of the season were affected by defoliation in all organs*

The stem was the dominant organ for carbohydrate content, accounting for 47% of the whole-tree TNC pool, leaves accounted for 16.5% of tree TNC and roots for 36.5%. These proportions were stable across treatments (Figure 3).

There was no significant effect of water stress on TNC pools in any organ (Table 3, Table S1 available as Supplementary Data at *Tree Physiology* Online). In contrast, defoliation had significant effects on carbohydrate pools in all organs and at the whole-tree level (Table 3, Figure 4a). Differences in TNC pools were only significant between undefoliated and 100% defoliated trees (and not for 50% defoliated trees). The whole-tree TNC pool was reduced by 36% in 100% defoliated trees compared with undefoliated trees (604.8 ± 173.1 vs 944.8 ± 129.3 g tree\(^{-1}\)).

Defoliation reduced not only TNC pools in 2010 needles through direct biomass reduction but also TNC pools in 2011 needles through a reduction in concentration (Table 3). One hundred percent defoliation reduced TNC in roots by 44% (219.9 ± 75.6 g tree\(^{-1}\)) in comparison with undefoliated trees (390.8 ± 72.9 g tree\(^{-1}\)) (Figure 4b). By contrast, TNC pools in stem sapwood were significantly higher in 50% defoliated trees than in the control trees and unchanged in 100% defoliated trees at the end of the growing season (Figure 4c). A similar trend was observed for SS and starch in stem sapwood (data not shown).

![Figure 1. Effects of defoliation level and water treatments on annual radial growth of *P. pinaster*. Dots with different letters correspond to significantly different mean values (Tukey’s tests, α = 0.05).](image1)

![Figure 2. Effects of defoliation and water stress on intra-annual stem growth (normalized circumference increment CI(d) (mm)).](image2)
In May, defoliation resulted in significantly lower TNC in stems for both 50% defoliated trees and 100% defoliated trees in comparison with control trees (respectively 407 ± 57 g tree\(^{-1}\) vs 581 ± 69 g tree\(^{-1}\) in control trees) \((F = 26.9, P < 0.0001)\). However by July, TNC pools were restored in both 50 and 100% defoliated trees (respectively

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**Figure 3.** Mean proportions of TNC pools in different organs of trees subjected to water stress (b) or in watered plots (a) and 0, 50 or 100% defoliation. The area of each pie is proportional to the TNC pool per tree (g tree\(^{-1}\)).

**Table 3.** Summary of two-way ANOVA results showing the effects of water treatment (WT, df = 1), defoliation rate (DR, df = 2) and their interaction (df = 2) on carbohydrate pools in *P. pinaster* trees 10 months after treatments were imposed (TNC, total non-structural carbohydrates; SS, soluble sugars).

<table>
<thead>
<tr>
<th>Resource pool (g tree(^{-1}))</th>
<th>WT</th>
<th></th>
<th>DR</th>
<th></th>
<th>WT × DR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Leaf SS</td>
<td>0.95</td>
<td>0.34</td>
<td>64.66</td>
<td>6.07 \times 10^{-9}***</td>
<td>0.33</td>
<td>0.72</td>
</tr>
<tr>
<td>Leaf starch</td>
<td>0.03</td>
<td>0.87</td>
<td>9.96</td>
<td>0.0012**</td>
<td>0.50</td>
<td>0.61</td>
</tr>
<tr>
<td>Leaf TNC</td>
<td>0.998</td>
<td>0.33</td>
<td>69.26</td>
<td>3.52 \times 10^{-9}***</td>
<td>0.33</td>
<td>0.72</td>
</tr>
<tr>
<td>Leaf 2010 TNC</td>
<td>1.81</td>
<td>0.19</td>
<td>256.59</td>
<td>7.12 \times 10^{-13}***</td>
<td>2.75</td>
<td>0.11</td>
</tr>
<tr>
<td>Leaf 2011 TNC</td>
<td>0.077</td>
<td>0.78</td>
<td>9.73</td>
<td>0.0054**</td>
<td>0.07</td>
<td>0.79</td>
</tr>
<tr>
<td>Phloem SS</td>
<td>0.24</td>
<td>0.63</td>
<td>1.70</td>
<td>0.21</td>
<td>0.08</td>
<td>0.92</td>
</tr>
<tr>
<td>Phloem starch</td>
<td>1.69</td>
<td>0.21</td>
<td>3.73</td>
<td>0.044*</td>
<td>0.24</td>
<td>0.79</td>
</tr>
<tr>
<td>Phloem TNC</td>
<td>1.96</td>
<td>0.17</td>
<td>0.16</td>
<td>0.69</td>
<td>0.07</td>
<td>0.93</td>
</tr>
<tr>
<td>Sapwood SS</td>
<td>0.12</td>
<td>0.74</td>
<td>6.38</td>
<td>0.008**</td>
<td>3.23</td>
<td>0.06</td>
</tr>
<tr>
<td>Sapwood starch</td>
<td>3.15</td>
<td>0.093</td>
<td>8.00</td>
<td>0.0033**</td>
<td>1.49</td>
<td>0.25</td>
</tr>
<tr>
<td>Sapwood TNC</td>
<td>0.0047</td>
<td>0.95</td>
<td>8.46</td>
<td>0.0026**</td>
<td>2.53</td>
<td>0.11</td>
</tr>
<tr>
<td>Root SS</td>
<td>4.42</td>
<td>0.05</td>
<td>4.47</td>
<td>0.027*</td>
<td>0.07</td>
<td>0.94</td>
</tr>
<tr>
<td>Root starch</td>
<td>0.0075</td>
<td>0.93</td>
<td>6.91</td>
<td>0.0053**</td>
<td>1.97</td>
<td>0.17</td>
</tr>
<tr>
<td>Root TNC</td>
<td>3.55</td>
<td>0.074</td>
<td>6.94</td>
<td>0.0051**</td>
<td>0.36</td>
<td>0.70</td>
</tr>
<tr>
<td>Total SS</td>
<td>0.71</td>
<td>0.41</td>
<td>10.2</td>
<td>0.0011**</td>
<td>1.33</td>
<td>0.29</td>
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<tr>
<td>Total starch</td>
<td>0.75</td>
<td>0.40</td>
<td>8.54</td>
<td>0.0025**</td>
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<td>0.51</td>
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<tr>
<td>Total TNC</td>
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<td>0.36</td>
<td>11.91</td>
<td>0.00039***</td>
<td>1.29</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*F*-value statistics were calculated with ANOVA at the tree level. *P* < 0.05; **P* < 0.01; ***P* < 0.001.
545 ± 62 and 377 ± 62 g tree⁻¹ vs 531 ± 42 g tree⁻¹ in control trees) (P > 0.05). Once again, water stress had no significant effect on stem carbohydrate pools during the growing season (P > 0.05).

Discussion

We found that the combination of defoliation and drought resulted in significantly reduced P. pinaster annual stem growth. The lack of any significant interaction between the two factors contradicts our initial hypothesis that defoliation would reduce the impact of water stress by reducing water losses and suggests an additive effect of the two stresses on tree growth. We also found that NSC in all organs were affected by defoliation but tree tolerance to water stress was not triggered. Stem NSC were maintained or increased at the end of the season, suggesting either an active maintenance of the hydraulic system or another limiting resource for tree growth under defoliation. Root carbohydrates were significantly reduced, which suggests a shift in the root carbon balance.

Additive effects of defoliation and water stress on P. pinaster annual stem growth

Annual stem growth was reduced by 88% in 100% defoliated trees in watered plots (no water stress). Significant stem growth losses ranging from 20 to 90% have been already reported in P. pinaster following 100% defoliation by PPM (Lemoine 1977, Markalas 1998, Arnaldo et al. 2010, Jacquet et al. 2012). The annual growth was not significantly reduced in 50% defoliated trees. This result contrasts with previous studies showing significant growth losses up to 50% for such PPM defoliation intensities and for trees of the same age (Arnaldo et al. 2010, Jacquet et al. 2012). This is consistent with the fact that responses to defoliation are complex and that you would not necessarily expect consistency between experiments in field trials. Furthermore, we suspect an effect of the previous-year history on the current-year tree responses.

In undefoliated trees, there was a significant drought-induced reduction of annual stem growth. On average, the radial growth in water-stressed trees was reduced by ~35%. Such a decrease in radial growth has been described for drought-prone forests throughout the world (Allen et al. 2010). More specifically, our results are consistent with a drought-induced growth decline of maritime pine forests in south-eastern Spain (Sánchez-Salgueiro et al. 2010). It has been reported that water availability, more than any other resources, determines the annual growth potential of individual trees (Bréda et al. 2006). Initially the most important impact of water stress on growth is via reduced turgor. Then, the reduction in soil water availability caused by water stress can decrease the carbon assimilation of trees via a stomatal regulation of leaf water potential and may also in turn cause a decline in radial growth (McDowell et al. 2008).

The combination of defoliation and drought resulted in significantly reduced P. pinaster annual stem growth, and the lack of any significant interaction between the two factors suggests an additive effect of the two stresses. In water-stressed trees, the annual radial growth was reduced from 6.03 cm year⁻¹ in control trees to 3.94 cm year⁻¹ in 100% defoliated trees. Water stress caused a 35% reduction in tree growth of non-defoliated trees. If we apply this estimated reduction to 100% defoliated trees, we should obtain a theoretical annual tree growth of 0.43 cm year⁻¹ under the hypothesis of additive defoliation and water stress effects, and this is very close to the observed annual radial growth of 0.56 cm year⁻¹. This is consistent with the results of a meta-analysis that showed larger impacts of defoliation on growth in water-stressed trees (Jactel et al. 2011) but contradicts our initial hypothesis of antagonist effects, i.e., that defoliation would reduce the impact of water stress on stem growth by reducing water losses through transpiration by the tree crown. Our results are also consistent with an ecology review reporting on the additive effects of...
harsh environmental conditions and insect herbivory on plant performance (Valladares et al. 2007) and with observational studies that show significantly larger defoliation effects on stem growth in low-productivity (low-nutrient) sites (Ovaska et al. 1993, Pinkard and Beadle 1998, Pinkard et al. 2007), suggesting that the effects of abiotic stresses and defoliation on tree growth are additive. However, these observations contrast with other reviews that predict antagonist effects of biotic and abiotic stresses on growth in woody plants (Hawkes and Sullivan 2001, Wise and Abrahamson 2007). Unfortunately, very few experiments have manipulated both water supply and defoliation to examine their combined effects on tree growth. Those rare experimental studies also provided contrasting results. Kolb et al. (1999) found higher stem biomass in potted Douglas-fir seedlings submitted to western spruce budworm defoliation under low- than under high-moisture conditions, which is compatible with antagonist effects of water stress and defoliation. In Eucalyptus globulus (Eyles et al. 2009, Quentin et al. 2012) and in red oak seedlings (McGraw et al. 1990), tree growth was unaffected by defoliation under different water treatments, which might be due to compensatory responses to defoliation and drought. One of the strengths of our study was that it examined the combined effects of water stress and defoliation on 10-year-old trees under field conditions. It may explain why partial defoliation does limit the impacts of water stress on those trees as they are sharing their water supply with undefoliated and fully defoliated trees. Antagonist effects of defoliation and water stress on tree growth may occur for younger and potted trees but might not exist for trees under stand conditions.

**Defoliation affects NSC pools but does not trigger tree tolerance to water stress**

Defoliation resulted in a net loss of stored carbohydrates due to needle consumption (Li et al. 2002) and a decrease in carbon fixation through reduced photosynthesis. In our study, artificial defoliation also resulted in the depletion of whole-tree TNC pools and more specifically in roots and foliage TNC pools. Such declines in carbohydrate reserves in both roots and remaining foliage have been observed following leaf removal (Hudgeons et al. 2007, Eyles et al. 2009). Those results are consistent with the view that defoliation effects on stem growth might be mediated by a shortage in carbohydrate storage (Vanderklein and Reich 1999). Despite this, we found that stem carbohydrates were only temporarily reduced by defoliation (in May 2011). This is consistent with the literature showing a short duration of carbon (source) limitation for stem growth even after severe defoliation (Palacio et al. 2008, 2011). For example, in evergreen conifers (Pinus cembra), a rapid replenishment of carbon stores following defoliation has been observed (Li et al. 2002, Roitto et al. 2003). At the end of the growing season, we found significantly higher stem TNC pools in 50% defoliated trees and unchanged pools in 100% defoliated trees compared with undefoliated trees. Furthermore, in 100% defoliated trees, stem growth was strongly affected whereas stem TNC were maintained for the second half of the season, suggesting that reduced stem growth was not dependent on local TNC storage. Similarly, higher stem TNC pools in 50% defoliated trees did not result in higher annual stem growth in comparison with control trees. These findings suggest that stem growth responses may not directly depend on changes in TNC stores. On one hand, maintenance (in 100% defoliated trees) and increase (in 50% defoliated trees) of carbohydrate pools could be considered as a consequence of other resource limitations (such as mineral) in tree physiological processes that lead to reduced consumption or even accumulation of carbon stores. Indeed, a characteristic of conifers is that they store nutrients such as nitrogen in old needles (Millard et al. 2001). Thus, artificial defoliation may have led to significant nitrogen shortage. But on the other hand, those results support the hypothesis that NSC may be an active carbon sink, crucial to maintaining hydraulic functioning in trees under water stress (Sala et al. 2012). Indeed, this hypothesis, especially under water-limited conditions, would explain why TNC pools were maintained prior to tree growth.

Furthermore, the large and significant decrease in carbohydrate pools in the roots of defoliated and water-stressed trees suggests a shift in the root carbon balance under defoliation. The decrease in carbohydrate supply under defoliation may not counterbalance the carbon use for mineral and water uptakes or a translocation to other tissues. Indeed, it is likely that localized carbohydrate consumption was maintained since root carbohydrates can be mobilized to increase mineral uptake (Eyles et al. 2009). The efficiency of soil mineral absorption in trees depends on both spatial extension and density of their root system (Levitt, 1980), and several carbon-demanding processes may have been involved to increase this absorption, such as enhanced root proliferation (Robinson 2001) or interactions with mycorrhizae (Marschner and Dell 1994).

**Conclusions**

Our results showed additive effects of defoliation and water stress on tree growth and contradict our first hypothesis of compensatory mechanisms. Even if NSC in all organs were affected by defoliation, tree tolerance to water stress was not triggered. We found that stem TNC were maintained or increased at the end of the season, suggesting either an active maintenance of the hydraulic system or another limiting resource for tree growth under defoliation. We also observed a significant decrease in root carbohydrates, which suggests a carbon use for increasing mineral and water uptakes.

As the occurrence of insect defoliation is likely to increase with climate change, our results suggest that such additive
detrimental effects will lead to a higher decline in forest productivity than expected from the consideration of only drought impacts.

As future research prospects, we think that the study of nitrogen and carbon coupling at the tree level under combined water stress and defoliation would add to the understanding of our results. We also suspect that after a certain point, severe drought would be a limiting factor and that both abiotic and biotic stresses could not be additive anymore. It would be crucial to determine that critical point.

**Supplementary data**

Supplementary material is available at *Tree Physiology* online.

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**Conflict of interest**

None declared.

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**References**


