

1 **Fast replenishment of initial carbon stores after defoliation by the pine**
2 **processionary moth and its relationship to the re-growth ability of trees**

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18

19 **Abstract**

20 Defoliation by herbivores may alter the source:sink balance of trees leading to transient decreases in
21 carbon (C) stores. When C stores are replenished concurrently with re-growth both processes may
22 compete, store formation proceeding at the expenses of growth. However, the interactions between both
23 processes are not fully understood. We investigated the effects of defoliation by the pine processionary
24 moth (PPM, *Thaumetopoea pityocampa* Dennis and Schiff.) on the non-structural carbohydrate (NSC)
25 and nitrogen (N) stores and the growth of *Pinus nigra* Arnold trees. Short-term effects were evaluated
26 immediately after a PPM outbreak and at the end of the first growing season in trees suffering a range of
27 defoliation damage. Long-term effects were explored by a 17-year-long PPM defoliation experiment,
28 with eleven years of repeated defoliation treatments followed by six years of recovery. Defoliation by
29 PPM was followed by transient NSC decreases, but trees were able to exceed initial NSC pools and
30 compensate growth in just one growing season. Such recovery was linked to increased foliage N.
31 Repeated severe defoliations decreased growth and survival of trees in the long-term, but trees increased
32 starch allocation to stems. Defoliation led to an accumulation of C storage compounds in *P. nigra* trees
33 irrespective of their ability to re-grow. In trees included in the short-term experiment, the accumulation of
34 stores proceeded concurrently with re-growth. However, the repeated severe defoliations included in our
35 long-term experiment impaired the growth of trees, surplus C being accumulated as stores. These results
36 indicate that, growth declines in pines defoliated by PPM are not due to C (source) limitation but may
37 respond to reduced sink strength of growing meristems due to defoliation and, thus, a decrease in C
38 allocation to growth.

39

40 **Keywords:** *Pinus nigra*, *Thaumetopoea pityocampa*, insect herbivory, non-structural carbohydrate,
41 nitrogen, storage allocation.

42

43 **Introduction**

44 Defoliation by herbivores reduces canopy leaf area causing a decrease in the net carbon (C) gain of trees
45 by current photosynthesis and altering the balance between C sinks and sources (Trumble et al., 1993).
46 This may lead to changes in C allocation patterns, C demands of growing sinks being supplied temporally
47 by C “stores” (Kozlowski 1992; Pinkard et al. 1998; Quentin et al. 2011), namely non-structural
48 carbohydrates (NSC) and lipids, which can be mobilized in the future to support growth or other plant
49 functions (Chapin et al. 1990). This broad definition of stores includes accumulation, reserve formation
50 and recycling (Chapin et al. 1990).

51 Depending on the severity, frequency and timing of damage, C stores may be decreased or even
52 depleted, and the growth of trees may become C source (photosynthetic) limited (Trumble et al. 1993).
53 This situation seems to be more dramatic in evergreen conifer trees, which store most of their C and
54 nitrogen (N) in the old foliage and hence may lose most of their C and N stores with defoliation (Li et al.
55 2002; Millard et al. 2001). Since the carboxylating enzyme Rubisco is one of the most important N
56 storage proteins in plants (Millard et al. 2007, 2010), the decrease in N stores after defoliation may also
57 impair the C-uptake ability of evergreen conifers after damage.

58 Multiple studies have shown that C stores of evergreen trees decrease soon after defoliation
59 (Ericsson et al. 1980; Li et al. 2002; Webb and Karchesy 1977). However, trees are able to compensate to
60 some degree for loss of foliage by changing allocation patterns favouring foliage production, increasing
61 the photosynthetic rate of surviving leaves and inducing changes in leaf morphology (Heichel and Turner
62 1976; Pinkard and Beadle 1998; Vanderklein and Reich 1999). Consequently, light defoliations do not
63 normally cause a decrease in NSC pools (Kolb et al. 1992; Tschaplinski and Blake 1994; Van der Heyden
64 and Stock 1995) and the few studies that have followed the evolution of NSC pools after moderate or
65 severe defoliations indicate C (source) limitation is only transient and of short duration (Palacio et al.
66 2008, 2011). In evergreen conifers, trees seem to be able to replenish their initial NSC pools after severe
67 defoliations rather soon (Li et al. 2002; Roitto et al. 2003), although long-term studies assessing their
68 recovery are lacking.

69 The ability of plants to increase C gain ability after defoliation has been suggested as a
70 mechanism to support re-growth and, ultimately, tree tolerance to defoliation (Eyles et al. 2009).
71 However, the observation that NSC pools are replenished concurrently with re-growth, i.e. throughout the
72 course of a growing season (Li et al. 2002; Palacio et al. 2008, 2011), poses the question as to what point

73 both processes interfere. According to the cost-benefit theory for plant storage (Chapin et al. 1990), the
74 replenishment of C stores after defoliation should compete with growth, if true reserve formation is
75 involved. Implicit in this idea is the assumption that plant growth is limited by C (source) availability and
76 that C allocation to storage can be regarded as a cause for decreased plant growth. Alternatively, C store
77 accumulation would proceed when acquisition exceeds demands (Chapin et al. 1990), once growth has
78 been impaired by defoliation. Identifying how growth recovery and C store replenishment interact is
79 crucial to understanding plant responses to disturbance and stress. However, to our knowledge, no
80 previous studies have directly assessed the relationship between both processes in defoliated trees.

81 The pine processionary moth (PPM), *Thaumetopoea pityocampa*, (Den. & Schiff.) is one of the
82 most severe insect pests affecting Mediterranean evergreen conifers such as *Pinus* and *Cedrus* species
83 (Masutti and Battisti 1990). PPM caterpillars can consume needles of any age during autumn and winter.
84 However, they feed preferentially on the old needles of trees, and defoliations are most intense during
85 winter when larvae are in the fourth and fifth instar (Démolin 1969). PPM larvae do not damage the buds,
86 which burst in spring (Guyon 1986). When larvae are fully developed, they abandon the tree, marching in
87 lines (hence their name) until they burrow in the ground where they turn into a chrysalis and then a moth,
88 after a period that can range from a few months to several years (Battisti 1988). Such variability
89 determines the population dynamics of this species, which, like many other foliage feeding insects shows
90 periodical outbreaks, with an average periodicity of 6 years (Battisti 1988), although the cycle is not
91 regular and it can exhibit sharp variations (Geri and Miller 1985). Defoliation by PPM has been shown to
92 decrease the growth and the reproductive abilities of pine species and may threaten the survival of drought-
93 stressed trees (Hódar et al. 2003; Kanat et al. 2005). C (source) limitation has been suggested as a cause
94 of such growth declines (Hódar et al. 2003), although no studies have evaluated the impact of PPM
95 damage on pine C stores.

96 The aim of this study was to evaluate the impact of PPM on the NSC and N stores of planted
97 *Pinus nigra* Arnold trees and their ability to replenish such stores in the short- (1 year) and the long-term
98 (i.e. six years, the time frame of an average PPM outbreak). We use mass-based concentrations of NSC
99 (namely soluble sugars and starch) as estimators of store formation and use (Chapin et al. 1990). We are,
100 however, aware that part of the NSC pools of plants may actually be sequestered and hence not readily
101 available for plant growth (Millard and Grelet, 2010). Consequently, C stores may be somewhat
102 overestimated in our analysis. We consider just NSC since, although lipids are important C storage

103 compounds for some conifers, NSC account for most of mobile C pools in pine (Li et al 2002). We
104 hypothesized that: i) Defoliation by the PPM will decrease NSC concentrations of trees causing a
105 transient C-limitation; ii) C stores will be soon replenished so that the initial C limitation will be
106 overcome in the course of a growing season; iii) Within the average time-frame of PPM outbreaks (six-
107 year periodicity), C stores of trees will be fully recovered by the end of a cycle. Also, we specifically
108 assessed the relationship between initial NSC stores and growth on the recovery of NSC reserves of *P.*
109 *nigra* trees after PPM outbreaks by using structural equation models (SEM). Since we expected trees not
110 to be limited by C (source) availability, our hypothesis was that the replenishment of carbohydrate stores
111 would not compete with the ability of trees to compensate growth after defoliation.

112

113 **Materials and methods**

114 *Species and study site*

115 *Pinus nigra* subsp. *nigra* Arn. trees were sampled in a plantation located near Mora de Rubielos, Teruel,
116 eastern Spain (40° 12' N, 0° 43' W, 1150 m s.n.m.). The trees were planted between 1968 and 1971 and
117 form a relatively open stand. Average diameter at breast height (DBH, measured at 1.3 m) and total tree
118 height were 8.0 ± 0.4 cm and 4.3 ± 0.1 m, respectively, at the beginning of the study (1992). The studied
119 *P. nigra* trees usually bear up to 3 to 4-year old needles. Climate in this area is Mediterranean with a
120 pronounced summer drought. The mean annual temperature in the study area is 12 °C and the total annual
121 rainfall is 436 mm based on monthly climatic data from the nearby “Mora de Rubielos” climatic station,
122 located ca. 6 km from the sampling site (40° 15' N, 0° 45' W, 1040 m, period 1992-2010). During the
123 study period, the precipitation recorded from April to June (mean = 144 mm) was low (49-117 mm) in
124 1993, 1994, 2001 and 2005.

125 The natural vegetation is dominated by *Quercus ilex* L. subsp. *ballota* (Desf.) Samp., *Quercus*
126 *coccifera* L. and *Juniperus* species. The soils are basic, nutrient-poor and developed on clays. Based on
127 historical records of PPM incidence in the area performed since the 1970s by the Spain and Aragón Forest
128 Services, no previous severe defoliation had affected the studied stand. A more detailed description of the
129 study site can be found in Hernández et al. (2005).

130

131 *Experimental design*

132 We combined observations after a natural PPM outbreak occurring in winter 2009-2010, with a 17-year-
133 long defoliation experiment conducted between 1992 and 2009, to assess the short- and long-term effects,
134 respectively, of PPM defoliations on the C stores and growth of trees and their subsequent recovery.
135 Short-term effects inform us about the mechanisms behind the recovery of trees while long-term data
136 illustrate the expected response of trees in the longer term under extreme defoliation scenarios.

137

138 SHORT-TERM EFFECTS: NATURAL PPM OUTBREAK IN WINTER 2009-2010.

139 In winter 2009-2010 a natural PPM outbreak affected the studied stand, leading to severe defoliation
140 (over 80% of the whole canopy) of some individuals. In April 2011, we randomly selected trees within
141 the stand not included in other defoliation experiments and that showed a wide range of crown defoliation
142 and related PPM incidence. We visually assessed the degree of needle loss (defoliation) of each sampled
143 tree by comparing them with five randomly selected reference trees showing no damage. These trees were
144 used as reference “control” trees to correct for observer bias in estimates of defoliation. Since percentage
145 estimates of crown defoliation vary among observers, all defoliation estimates were made by the same
146 person (JJC). The mean diameter of sampled trees measured at 1.3 m was 16.97 ± 0.32 cm (mean \pm SE),
147 whereas the mean age, estimated at 1.3 m by counting rings in radial cores, was 36 ± 1 years. The mean
148 distance between sampled trees ranged between 5 and 10 m.

149

150 LONG-TERM EFFECTS: DEFOLIATION EXPERIMENT

151 In 1992, we randomly selected 20 trees within the study stand and allocated them to two different
152 defoliation treatments: 100% defoliation and control (undefoliated). Although frequent complete
153 defoliations by PPM are rare in nature, we aimed at simulating extreme defoliation scenarios to detect
154 mid- to long-term responses in growth to severe needle loss and to discern the time required by trees to
155 recover growth levels similar to those observed before defoliations started. All trees were tagged and their
156 height and DBH measured with tapes and ladders to account for initial (i.e. not due to treatments) tree
157 variability in size. Treatments were applied for 11 years, between 1993 and 2003, by transposing PPM
158 nests to trees included in the “defoliation” treatment. Defoliation damage was checked regularly
159 throughout autumn and winter. If larvae hatched from transplanted nests were not enough to cause a
160 complete defoliation of trees, more nests were transplanted from nearby affected trees. If still, canopy
161 defoliation was not complete, needles of undefoliated branches were gradually clipped with scissors,

162 avoiding clipping complete branches and buds were left intact. This procedure aimed at achieving gradual
163 but complete (and hence comparable) canopy defoliation, mimicking natural (yet extremely severe) PPM
164 outbreaks.

165 Treatments were applied in autumn and winter for two consecutive years followed by a year of
166 recovery. Consequently, defoliations were applied in 1993 – 1994, 1996 – 1997, 1999 – 2000 and 2002 –
167 2003, and trees were released from defoliation in years 1995, 1998 and 2001. From 2004 onwards, trees
168 were left to recover until November 2009 (6 years later), when they were felled and harvested for
169 analysis. Three of the initial 10 trees included in the defoliation treatment died during the experiment and,
170 consequently, our sample size was reduced to $n = 7$ for defoliated trees.

171

172 *Growth measurements*

173 Short-term effects of PPM on growth were assessed by measuring the needle production, branch length
174 and radial growth (earlywood, latewood and total current-year wood ring width) of trees in September
175 2011 (i.e. one growing season after the PPM attack). Primary growth was estimated by randomly
176 collecting three current-year branches from the upper third, southern-oriented and light-exposed side of
177 the crowns of each tree. All sampled branches were healthy and had grown in 2011 and thus contained
178 needles formed in that year. We measured the length of the shoot formed in 2011 (i.e. lateral branch
179 growth) and averaged the individual branch values per tree. Then, we measured the average needle
180 biomass production in 2011 of each tree by drying (in the oven at 60°C for 72 hours) and weighing all the
181 needles formed in 2011 of the different branches harvested.

182 Secondary growth was measured in two radial cores per tree, taken at 1.3 m (breast height) using
183 a Pressler increment borer. Wood samples were carefully cut and visually cross-dated to measure the
184 width of the 2011 tree-ring. Once dated, we measured the earlywood and latewood widths to the nearest
185 0.01 mm using a binocular scope and a LINTAB measuring device (Rinntech, Heidelberg, Germany).
186 Total tree-ring width was calculated as the sum of the earlywood and latewood widths. Values of the two
187 radii were averaged to obtain annual estimates of radial growth per tree.

188 The growth of trees included in the long-term experiment was monitored annually by measuring
189 the height and DBH of each tree in November when both types of growth have been completed.

190 Secondary growth was measured as in the trees included in the short term experiment, by collecting two

191 cores per tree at 1.3 m. Wood cores were subsequently sanded and visually cross-dated, and the accuracy
192 of visual cross-dating checked with the program COFECHA (Holmes 1983).

193

194 *Plant harvest*

195 Only needles and main stem wood were considered in the analyses. These fractions (particularly needles)
196 account for the largest proportion of total NSC pools in pine trees and, consequently, are most affected by
197 defoliations (Li et al. 2002). For the short-term experiment, needle and stem wood samples were obtained
198 from marked trees in April 2011, prior to bud burst, and September 2011, at the end of the growing
199 season, when trees had completed both their primary and radial growth. All samples were collected
200 between 10:00 and 13:00 h to avoid diurnal variability in carbohydrate concentrations. Current-year
201 needles were collected from three current-year branches from the upper third, southern-oriented and light-
202 exposed side of the crowns of each tree. In September 2011, these were the same branches used for
203 primary growth measurements (see above). Stem wood samples were obtained from two radial cores per
204 tree, taken at 1.3 m using a Pressler increment borer. After collection in the field, needle and wood
205 samples were taken to the laboratory in a portable cooler. Needles were dried in the oven at 60°C for 72
206 hours. Portions of current-year tree-rings were separated from each core using a razor blade. Wood
207 samples were subsequently frozen and stored at -20 °C until freeze-dried. All dried samples were
208 weighted and milled to a fine powder in a ball mill (Retsch Mixer MM301, Leeds, UK) prior to chemical
209 analyses.

210 Plant samples for the long-term experiment were collected from the trees felled at the end of the
211 experiment in November 2009, when primary and secondary growth had terminated and trees were
212 dormant. From each tree, we collected two slices of the stem at 1.3m height plus needle samples of the
213 youngest 4 cohorts from sun-exposed top branches. All samples were collected between 10:00 and 13:00
214 h, kept in a cooler until transported to the lab and then frozen at -20 °C until processing. Since trees were
215 relatively young, most of the stem wood was sapwood. Samples of stem sapwood were hence collected
216 from different positions within each slice and divided in two sections for chemical analyses: an outer
217 section, including the youngest rings formed between 2004 and 2009, and an inner section with the oldest
218 rings (formed between 1996 and 2003). All samples were freeze-dried, weighted and milled to a fine
219 powder in a ball mill (Retsch Mixer MM301, Leeds, UK) prior to chemical analyses.

220

221 *Chemical analyses*

222 Total C and N mass-based concentrations were analyzed with an elemental analyzer (Elementar
223 VarioMAX N/CM, Hanau, Germany). Soluble sugars (SS) were extracted with 80% (v/v) ethanol and
224 their concentration determined colorimetrically, using the phenol-sulphuric method of Dubois et al.
225 (1956) as modified by Buysse and Merckx (1993). Starch and complex sugars remaining in the
226 undissolved pellet after ethanol extractions were enzymatically reduced to glucose and analyzed as
227 described in Palacio et al. (2007). Non-structural carbohydrates measured after ethanol extraction are
228 referred to as soluble sugars and carbohydrates measured after enzymatic digestion are referred to as
229 starch. Both are expressed in glucose equivalents. The sum of SS and starch is referred to as total non-
230 structural carbohydrates (NSC). NSC pools of current-year needles included in the short-term experiment
231 and collected in September 2011 were calculated as the product between the average current-year needle
232 biomass per branch and the NSC concentrations of current-year needles.

233

234 *Statistical analyses*

235 Short-term effects of PPM defoliation on the SS, starch, NSC and N concentrations and the C:N ratio of
236 needles and sapwood of *P. nigra* trees in April and September 2011 and on the starch, NSC and N pools
237 of needles in September 2011 were analyzed by univariate General Linear Models (GLMs, Least Squares
238 fit) with “defoliation” as a fixed factor and the “DBH of the tree in April” as a covariate to account for
239 tree individual variability in size. The same GLM was used to evaluate the short-term effects of PPM
240 defoliation on the growth of trees, i.e. current-year needle biomass per branch, lateral branch length, and
241 earlywood (EW), latewood (LW) and total current-year wood ring width as measured in September 2011
242 in the same trees.

243 Long-term effects of defoliation by PPM on the SS, starch, NSC and N concentrations and the
244 C:N ratio of needles and sapwood of *P. nigra* trees were analyzed by GLMs with “defoliation treatment”
245 (defoliated / control) and “cohort” as fixed factors and the “DBH of trees in 1992”, the year prior to the
246 first application of treatments, as a covariate. Four different cohorts of needles were considered, while in
247 stems, sapwood was divided in the two 1996-2003 and 2004-2009 segments. Long-term defoliation
248 effects on the growth of trees were evaluated by GLMs with “defoliation treatment” as a fixed factor and
249 the “DBH of trees in 1992” plus the “height of trees in 1992” as covariates. Response variables were the
250 DBH, height increment and total current-year wood ring width of trees on the first year after the

251 application of treatments (1993), the last year of the application of treatments (2003), the year of harvest
252 (2009), and the average DBH, height increments and annual wood ring width values for the period of
253 defoliations (1993-2003) and recovery (2003-2009). When variables were not normally distributed, they
254 were angularly transformed ($\arctg(x^{0.5})$) to meet the requirements of GLMs. All GLM and regression
255 analyses were run in JMP 8.0 (SAS Institute Inc., Cary, 1989-2007).

256 The relationship between store replenishment and re-growth ability of trees was studied by using
257 data from the short-term experiment. We followed two different methods: correlations and structural
258 equation modeling (SEM). In the first approach, the difference between April and September NSC
259 concentrations in leaves and stem sapwood were correlated against the different growth indicators (needle
260 biomass productivity, branch length growth and wood ring width increment) by Pearson correlation
261 analyses. Correlations were run in SPSS 15.0. (SPSS for Windows, Chicago, 2009).

262 Secondly, SEM was used to evaluate the more complex relationships between defoliation by
263 PPM, N and NSC concentrations in April, the difference between April and September NSC
264 concentrations (as an indicator of store replenishment) and growth. SEM is a robust tool to unravel
265 multivariate relationships between plant traits, as it enables disentangling direct and indirect effects
266 among variables (Shipley 2004). We first built a theoretical model including the logical relationships
267 between our variables based on previous knowledge. In our model defoliation was directly related to NSC
268 and N concentrations in April, owing to the well-documented immediate effects of defoliation on N and
269 NSC stores (Ericsson et al. 1980; Tschaplinski and Blake 1994; Webb and Karchesy 1977). We also
270 considered defoliation to be directly related to the growth attained at the end of summer, to account for
271 other effects of defoliation on plant growth different to the impact on NSC and N availability. NSC
272 concentrations in April were logically related to store replenishment in September, but also to growth,
273 since pine trees are known to invest both stored NSC and new photoassimilates into new growth (von
274 Felten et al. 2007). The concentration of N in April was also directly related to growth and the difference
275 between April and September NSC concentrations, owing to the close relationship between total N and
276 Rubisco concentrations in leaves and, hence, the carboxylation potential of trees (Millard et al. 2007).
277 Finally, growth attained throughout the growing season was related to the difference between April and
278 September NSC concentrations as a way to explore the relationship between NSC accumulation (store
279 replenishment) and growth after defoliation.

280 We fitted our theoretical model using SEM to six different data sets, depending on the origin of
281 NSC concentrations (needles or stem sapwood) and the variable used as a proxy for tree growth (needle
282 biomass production, branch length growth or current-year wood ring width increment). SEM estimation
283 followed the maximum likelihood method (Arbuckle 2009). The goodness of fit of each model was
284 evaluated by a combination of statistics and fit indexes including: the chi-square and its probability level,
285 the root mean square error of approximation (RMSEA), the root mean square residual (RMR), the
286 Goodness-of-Fit Index (GFI), the Adjusted Goodness-of-Fit Index (AGFI), the Normed Fit Index (NFI)
287 and the Akaike Information Criterion (AIC). Non-significant chi-square statistics indicate an acceptable
288 model fit, while values close to zero for the RMSEA and RMR indexes and values close to one for the
289 probability level, GFI, AGFI, and NFI statistics indicate consistency between the theoretical and
290 evaluated models. Finally, the AIC is an index used to compare different models: the most parsimonious
291 models showing the lowest AIC values. Since different indices follow different approximations and hence
292 reflect different aspects of model fit, the use of a combination of statistics and indexes provides a robust
293 tool to evaluate the fit of models generated by SEM (Jöreskog 1993). SEMs were performed in Amos
294 18.0.

295

296 **Results**

297 *Short-term effects of PPM defoliation on N and NSC concentrations*

298 The mean defoliation of sampled trees was 42 ± 5 %. The defoliation intensity was a good surrogate of
299 PPM incidence because canopy defoliation and the number of PPM nests counted per tree (mean 3 ± 1
300 nests tree⁻¹) were strongly related ($r = 0.68$, $P < 0.001$).

301 Defoliation by PPM had a strong immediate effect on the NSC of *P. nigra* trees in the needles
302 and a weak impact on the NSC accumulated in stem sapwood and the needle N concentrations. Trees
303 harvested soon after the end of PPM defoliation but before bud break, in April 2011, showed decreased
304 non-structural carbohydrate concentrations (including SS, starch and NSC) in both the needles and stems
305 as defoliation increased (Fig. 1; Table 1). On the contrary, N concentrations in old needles increased
306 significantly with defoliation, and hence trees with higher needle loss showed significantly lower C:N
307 ratios in their needles (Fig. 1; Table 1).

308

309 *Recovery of NSC stores and growth after one growing season*

310 At the end of the growing season (September 2011), trees included in the short-term analysis showed
311 significantly higher NSC concentrations and pools in current-year needles with increasing defoliation (Fig
312 2, Table 1). This indicates trees not only replenished their C stores in just one growing season, but the
313 refilling of stores was proportional to defoliation intensity, more defoliated trees showing increased NSC
314 concentrations and pools at the end of summer (Fig. 2). Patterns for N were also highly significant, N
315 concentrations and pools increasing and C:N ratios decreasing with damage (Fig 2, Table 1). In stems,
316 defoliation effects were not significant for any of the chemical variables studied (Table 1). Similarly,
317 GLMs showed there was a significant positive effect of PPM defoliation on branch growth ($F = 11.153$,
318 $d.f. = 1$, $P = 0.002$) and foliage production ($F = 17.369$, $d.f. = 1$, $P < 0.001$), trees with higher defoliation
319 damage showed increased branch growth and higher current-year needle biomass (Fig. 2). This also
320 explains the recovery of needle NSC and N pools. Defoliation effects were not significant for any
321 indicator of secondary growth (EW width: $P = 0.579$, LW width: $P = 0.311$, tree-ring width: $P = 0.464$,
322 Fig 2).

323

324 *Long-term effects of PPM defoliations on NSC and N concentrations and growth*

325 There were significant differences in the NSC and N concentrations and the C:N ratio of the different
326 cohorts of tree rings and needles analyzed (Table 2). In sapwood, the outermost rings, i.e. the youngest
327 ones, showed higher concentrations and sharper variations of NSC and N than older rings. This indicates
328 NSC and N was preferentially stored and remobilized from younger rings within the sapwood of *P. nigra*
329 trees. Contrastingly, older needles stored more NSC than younger cohorts (Table 2). For N, old cohorts of
330 needles of defoliated trees showed lower concentrations while in control trees the trend was reversed
331 (Table 2).

332 Differences between treatments showed that defoliated trees accumulated more NSC in their
333 sapwood and more N in both their sapwood and needles than control trees, consequently, the C:N ratio of
334 defoliated trees was also lower (Table 2). Starch needle concentrations seemed to follow an opposite
335 trend, decreasing in defoliated trees as compared to controls, although effects were weak due to the low
336 replication ($n = 3$) and not significant for needle SS and NSC concentrations (Table 2). Taken together,
337 these results seem to indicate a shift from needle to stem accumulation of NSC in response to long-term
338 defoliation treatments. Increased NSC (in sapwood) and N status of defoliated trees six years after the last
339 defoliation event indicates that these trees may reach the next PPM population outbreak (which have an

340 average periodicity of six years) with more C and N stores than non-defoliated trees (Table 2). The effects
341 on NSC in sapwood stood for trees subjected to similar treatments but with nine years of recovery (data
342 not shown).

343 The analysis of long-term effects on growth showed defoliation treatments significantly
344 decreased height and diameter growth of trees and the width of rings formed after PPM defoliations
345 (Table 3). These effects were already significant on the first year after defoliations started (1993, $F =$
346 41.90 , $d.f. = 1$, $P < 0.001$ for tree-ring width), and they remained until the end of the recovery period
347 (2009), six years after the completion of treatments, except for tree-ring width which did not show
348 significant differences in 2009 (Table 3). Long-term defoliation by PPM had also an important effect on
349 the survival of trees, and 30% of the ten initial individuals died. These data will be considered elsewhere
350 (Camarero and R. Hernández, unpublished results).

351

352 *Relationship between stores replenishment and growth after defoliation*

353 The six models obtained with SEM, built from the different data sets available, provided a reasonably
354 good fit to our theoretical model (Table 4). However, of all models explored, the model which included
355 the NSC data of sapwood and the width of the current year tree ring as a surrogate of tree growth (number
356 6), showed the best fit (Table 4) and hence was the one selected for representation (Fig. 3). Nevertheless,
357 provided there was considerable and meaningful variation in the significance of the different relationships
358 of the models obtained with different data sets (Fig. 3, Table 5), all models will be considered in the
359 discussion of results.

360 According to all SEMs obtained and in agreement with the results of GLMs, defoliation had a
361 significant immediate positive effect on foliage N concentrations and a negative one on sapwood and
362 needle NSC concentrations (Fig. 3, Table 5). Not surprisingly, NSC concentrations in spring were
363 negatively related to the replenishment of NSC stores at the end of the growth season (measured as the
364 difference between September and April NSC concentrations) in all models (Fig. 3, Table 5). However,
365 they were unrelated to any measurement of growth included in the analyses (Fig. 3, Table 5). Foliage N
366 concentrations in April were unrelated to the replenishment of sapwood NSC (Fig. 3), but they were
367 strongly and positively related to the increase in needle NSC concentrations throughout the growing
368 season (see data sets 1-3 in Table 5). Similarly, N concentrations did not show any effect on stem wood

369 growth (Fig. 3), but they were significantly and positively related to needle biomass production and
370 branch length increment (Table 5).

371 Direct effects of defoliation on growth were not significant for any growth indicator measured,
372 and only positive indirect effects were recorded for leaf biomass production and branch length growth,
373 through the aforementioned impact on N concentrations (Table 5). For wood growth, defoliation had no
374 significant effect whatsoever, which agrees with our results of GLMs for the short-term experiments (Fig.
375 2).

376 The growth attained at the end of summer by defoliated trees was unrelated to NSC refilling
377 when foliage production and tree-ring width were used as indicators of growth (Fig 3; Table 5). Similarly,
378 when the difference in the NSC status of April and September was directly related to growth parameters,
379 correlations rendered no significant results ($P > 0.05$; data not shown), indicating both processes were
380 unrelated. However, when branch length growth was used as a proxy for tree growth in SEMs, it was
381 significantly and negatively related to the level of NSC accumulated in both needles and sapwood
382 throughout the growing season (Table 5). Taken together, these results indicate NSC accumulation
383 proceeded even when trees were re-growing, although branches that grew more in length had less surplus
384 of NSC for accumulation.

385

386 **Discussion**

387 *Transient C limitation after PPM defoliation*

388 Defoliation by PPM decreased the NSC concentrations of trees leading to a short-term C (source)
389 limitation. The observed decrease in SS, starch and NSC concentrations proportional to defoliation in
390 April indicates trees had already started remobilizing C stores from their old needles to recover from
391 damage before bud burst. This does not seem to be the case for N, which showed an opposite trend,
392 although increases were very moderate (see Fig. 2). However, in accordance with our hypotheses, such C
393 limitation was only transient and *P. nigra* trees were able to replenish their NSC stores in just one
394 growing season. Similarly, trees subjected to extreme repeated defoliation events were able to compensate
395 C losses in the course of an average PPM outbreak cycle. This may enhance their chances to survive
396 subsequent defoliations. Altogether, these results seem to indicate reported (Hódar et al. 2003; Kanat et
397 al. 2005) and observed (Table 2) declines in tree growth and/or reproduction after PPM attacks are not
398 caused by a C (source) limitation.

399 The fast recovery of NSC concentrations in our experiment responded to a shift in biomass
400 allocation to foliage, as indicated by the significantly greater biomass of current year needles in more
401 severely defoliated trees (Fig. 3). However, compensatory increases in the photosynthesis of trees may
402 have also contributed to the recovery of NSC concentrations (Eyles et al. 2011; Pinkard and Beadle 1998;
403 Pinkard et al. 1998; Vanderklein and Reich 1999). According to our results, increased foliage N (hence
404 Rubisco) concentrations could be partly responsible of the fast recovery of the NSC status through an up-
405 regulation of the photosynthetic ability of trees (Hoogesteger and Karlsson 1992). Although we did not
406 measure the photosynthetic rate of trees in this study, the SEM analysis indicated a strong positive effect
407 of needle N concentrations in April on foliage NSC replenishment in September (Table 5). Also, needle N
408 concentration increased with defoliation immediately after damage (Fig. 1) and the situation persisted at
409 the end of the growth season (Fig. 2) and even six years after the attacks (Table 2). Similarly, Hódar et al.
410 (2004) found increased N concentrations in the needles of pine trees defoliated by the PPM.

411 Increased sink strength after defoliation (Stitt et al. 1991) could have also contributed to up-
412 regulate photosynthesis. Pinkard et al. (2007) found compensatory increases in the photosynthetic rate of
413 defoliated *Eucalyptus globulus* trees were positively related to the level of damage, which they attributed
414 to a regulation of photosynthetic responses by increased sink strength. Similarly, we found the recovery
415 of NSC pools and concentrations of trees to be proportional to damage (Fig. 2). Recent advances indicate
416 leaf starch and sucrose concentrations can act as regulators of photosynthesis, accumulation of
417 carbohydrates in leaves leading to a down-regulation of the photosynthetic rate (Kasai 2008).

418 Interestingly, we found no relationship between the NSC status of trees soon after defoliation
419 and their ability to re-grow (Fig. 4, Table 5). This may indicate the re-growth of defoliated *P. nigra* trees
420 was primarily supplied by current assimilation. Our results for the short-term experiment show needle
421 NSC concentrations were more sensitive to defoliation than stem sapwood ones. Also, previous studies
422 indicate early twig growth of pine trees largely depends on current year photosynthates (Hansen and Beck
423 1990, 1994; von Felten et al. 2007). Defoliation may increase the dependence of twig growth on current
424 photosynthesis in pine. Indeed, in our study, foliage N concentrations in April were significantly and
425 positively related to leaf biomass production and branch length increment (Table 5), suggesting current
426 photosynthesis was more important than stores in leaf biomass and primary growth recovery of *P. nigra*
427 trees. The lack of a similar response in radial growth (Fig. 4, Table 5) could be attributed to the

428 observation that wood formation in pines is supported by a mixture of stored and current-year C (von
429 Felten et al. 2007).

430

431 *Defoliation effects on growth*

432 We observed no significant short-term effects of defoliation on stem wood growth and even significant
433 positive effects on leaf biomass production and branch length growth (Fig 3). These results seem to
434 contradict previous reports of decreased radial and height growth on pine trees after PPM attacks (Hóðar
435 et al. 2003; Kanat et al. 2005) and our own results for the long-term defoliation experiment (Table 2). The
436 explanation to these apparent discrepancies may lay in the different approaches followed. While most
437 previous studies, and also our long-term experience, compared undefoliated trees with severely defoliated
438 ones (Kanat et al. 2005), trees in our short-term experiment were selected over a range of damage from 0
439 to 100 % defoliation, so that the number of trees with very severe damage (above 80%) was low. Hóðar et
440 al. (2003) analyzed the impact of PPM defoliation intensity on the growth rate of the leader shoots of *P.*
441 *sylvestris* and found that increased intensities of defoliation led to progressive decreases in shoot growth
442 rate. Although no direct statistical comparison between control and defoliated trees subjected to different
443 intensities of damage was performed, their results indicate that only those trees subjected to high
444 defoliation intensities show markedly reduced growth as compared to control trees (see Fig. 1 in Hóðar et
445 al. 2003). Consequently, when comparing control and defoliated trees, only severe defoliation treatments
446 seem to produce significant different abilities in trees to compensate defoliation damage. For pine trees,
447 removal of up to 80 % of leaves has been shown to have no significant impact on the lateral branch
448 growth of trees (Handa et al. 2005). Consequently, most of our observations in the short-term experiment
449 may have fallen below the level of damage for compensation growth to be detected, leading to the
450 observed results.

451 In any case, the results from our short-term experiment indicate that *P. nigra* trees are able to
452 recover efficiently from moderate (and punctual) PPM defoliations, and illustrate some of the
453 mechanisms used to achieve such recovery (see above). Contrastingly, our long-term experiment provides
454 information about the potential impacts of severe and repeated PPM defoliations on *P. nigra* growth and
455 NSC stores. Our results demonstrate that although repeated severe defoliation by PPM can have a strong
456 impact on the growth and survival of *P. nigra* trees, 66% of the trees were able to survive the extreme
457 treatments imposed and they did not show C starvation.

458

459 *Interactions between NSC accumulation and growth after defoliation*

460 Defoliation led to increased accumulation of C storage compounds in *P. nigra* trees in both the short- and
461 the long-term while re-growth was only maintained under moderate damage. Correlation analyses and
462 SEM indicated that, when damage was moderate, as in our short-term experiment, C-store accumulation
463 and re-growth after defoliation were generally unrelated. The only component of growth that seemed to
464 have a negative impact on NSC accumulation was branch elongation, with branches that grew more
465 showing smaller surpluses of NSC for accumulation (Table 5). Indeed, in our short-term experiment, trees
466 with higher defoliation levels showed both higher NSC pools and concentrations and re-growth than trees
467 with less damage (Figs. 2 and 3). These results are in agreement with our third hypothesis.

468 However, in trees subjected to extreme and recurrent defoliations (such as our long-term
469 treatments), 30% of trees died and, for those trees that survived, growth was not recovered to the same
470 extent as NSC concentrations. The increase in stem NSC concentrations indicates these trees were not
471 limited by C availability (Hoch et al. 2003; Körner 2003). What, then, limited their growth? We suggest
472 repeated extreme defoliations reduced the sink strength of growing meristems (sink limitation), so that the
473 surplus of fixed carbohydrates not invested in growth accumulated as storage compounds. In line with
474 this suggestion, indole-3-acetic acid (IAA), which favours assimilate import into sink organs (Darussalam
475 et al. 1998), has been shown to decrease near the cambial region of pruned *P. sylvestris* trees (Sundberg et
476 al. 1993), and this has been related to reduced radial growth after pruning (Thomas et al. 2006).

477 In conclusion, our results show *P. nigra* trees respond to defoliation by accumulating
478 carbohydrate storage compounds. Defoliation by PPM caused only a transient C (source) limitation in *P.*
479 *nigra* trees and after just one growing season trees were even able to over-compensate initial NSC losses.
480 Also, within the average periodicity of PPM outbreaks, trees are able to fully recover their initial NSC
481 concentrations. When defoliation is moderate, we suggest the sink strength of growing meristems is not
482 decreased and the compensatory increase in the photosynthetic ability of trees enables them to recover
483 both growth and initial NSC status. However, when damage is extreme and recurrent, the sink strength of
484 growth processes may be reduced leading to decreased tree growth and surplus sugars being accumulated
485 as NSC.

486

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496

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- 618

619 **Figure legends**

620 **Fig. 1** Relationship between the percentage of canopy defoliation by the PPM and the NSC
621 concentrations of the needles and stems and the N concentrations and C:N ratios of the current-year
622 needles of *P. nigra* trees included in the short-term experiment and measured in April 2011. Results of
623 linear regression analyses are shown separately in each chart

624
625 **Fig. 2** Relationship between the percentage of canopy defoliation by the PPM in April 2011 and the NSC
626 and N concentrations and pools and the C:N ratio of the current-year needles of *P. nigra* trees included in
627 the short-term experiment and measured in September 2011. Regression lines are shown only when
628 significant ($P < 0.05$).

629
630 **Fig. 3** Relationship between the percentage of canopy defoliation by the PPM in April 2011 and the
631 branch growth, foliage production (biomass of current-year needles) and radial increment (tree-ring width
632 of the current year) of *P. nigra* trees included in the short-term experiment and measured in September
633 2011. Regression lines are shown only when significant ($P < 0.05$).

634
635 **Fig. 4** Structural equation model showing the relationships between the non-structural carbohydrate
636 (NSC) and nitrogen (N) concentrations and the growth of *P. nigra* trees included in the short-term
637 experiment of PPM defoliation. Data used for NSC and N concentrations belong to sapwood and needles,
638 respectively; while “growth” represents current-year tree-ring width ($n = 50$). These variables were
639 selected following model 6, the one with the best goodness of fit (see Table 4). Results for models
640 considering NSC in needles and branch growth processes are summarized in Table 5. Solid and dashed
641 arrows indicate positive and negative effects, respectively. Arrow widths are proportional to the
642 magnitude of standardized path coefficients. ‘n.s.’ indicates paths were non-significant at $\alpha = 0.05$.
643 Unexplained variance, i.e. error terms, of each observed variable is indicated by arrows located near
644 response variables. The proportion of explained variance (R^2) is interpreted similarly to a regression
645 analysis

646

647 **Table 1.** Statistics assessing the effects of defoliation on the NSC and N concentrations and pools of trees
 648 harvested in April 2011 (just after PPM defoliation) and in September 2011 (one growing season after
 649 PPM defoliation). Differences due to defoliation intensity in starch, soluble sugars (SS), total non-
 650 structural carbohydrate (NSC) and total nitrogen (N) and carbon (C) concentrations and the C:N ratios
 651 were assessed in needles and stems by GLMs. The model comprised “defoliation” as a fixed factor and
 652 the DBH at the beginning of the experiment as a covariate. Only “Defoliation” effects are shown.
 653 Significant effects ($P < 0.05$) are shown in bold, *d.f.* = 1 in all cases.

Variable	April 2011				September 2011			
	Needles		Stems		Needles		Stems	
	<i>F</i>	<i>P</i> -value	<i>F</i>	<i>P</i> -value	<i>F</i>	<i>P</i> -value	<i>F</i>	<i>P</i> -value
Concentrations								
SS	7.14	0.010	0.56	0.457	3.89	0.055	2.03	0.161
Starch	42.76	< 0.001	5.60	0.022	4.68	0.036	3.39	0.071
NSC	42.51	< 0.001	5.58	0.022	5.53	0.023	1.03	0.316
Total N	8.09	0.007	-	-	22.24	< 0.001	-	-
C:N	8.27	0.006	-	-	25.03	< 0.001	-	-
Pools								
SS	-	-	-	-	19.70	< 0.001	-	-
Starch	-	-	-	-	21.81	< 0.001	-	-
NSC	-	-	-	-	23.16	< 0.001	-	-
Total N	-	-	-	-	21.47	< 0.001	-	-

654
 655

656 **Table 2.** Soluble sugars (SS), starch, total non-structural carbohydrate (NSC), nitrogen (N) concentrations
 657 and C:N ratios in the older (1996-2003 rings) and younger (2003-2009 rings) sections of the sapwood and
 658 the three youngest cohorts of needles of *P. nigra* trees defoliated by the PPM in the long-term experiment,
 659 plus summary statistics of GLMs analyzing the differences between treatments and cohorts. Only fixed
 660 factor effects are shown (their interaction was not significant in any case). Values are means, while
 661 standard errors are indicated in parentheses. For statistics, values are *F* ratios, while *P*-values are
 662 indicated in parentheses, *d.f.* = 1 in all cases. Significant (*P*<0.05) factor effects are indicated in bold. See
 663 materials and methods for further details.

Fraction	Control			Defoliated			Statistics	
Sapwood	1996-2003 rings	2003-2009 rings	1996-2003 rings	2003-2009 rings	Treat.	Cohort		
SS	0.35	0.57	0.44	0.79	14.02	52.01		
(%)	(0.03)	(0.02)	(0.04)	(0.08)	(<0.001)	(<0.001)		
Starch	0.67	0.73	0.65	0.83	0.90	7.27		
(%)	(0.04)	(0.03)	(0.02)	(0.07)	(0.350)	(0.011)		
NSC	1.02	1.30	1.09	1.62	6.48	32.726		
(%)	(0.07)	(0.05)	(0.05)	(0.12)	(0.016)	(<0.001)		
Total N	0.02	0.03	0.04	0.05	41.84	11.15		
(%)	(0.002)	(0.003)	(0.002)	(0.004)	(<0.001)	(0.002)		
C:N	2233.19	1806.59	1385.78	1073.68	31.76	7.01		
	(176.10)	(164.95)	(67.52)	(78.13)	(<0.001)	(0.013)		

	Control			Defoliated			Statistics	
Needles	0-yr	1-yr	2-yr	0-yr	1-yr	2-yr	Treat.	Cohort
SS	4.66	5.13	5.41	5.24	5.28	5.56	0.05	2.10
(%)	(0.31)	(0.43)	(0.45)	(0.19)	(0.25)	(0.28)	(0.825)	(0.161)
Starch	3.92	4.53	4.31	4.08	3.77	4.00	5.63	1.59
(%)	(0.25)	(0.52)	(0.43)	(0.13)	(0.21)	(0.17)	(0.026)	(0.221)
NSC	8.56	9.66	9.72	9.31	9.05	9.56	1.40	2.44
(%)	(0.54)	(0.93)	(0.85)	(0.11)	(0.05)	(0.14)	(0.249)	(0.132)
Total N	0.81	0.83	0.89	1.04	0.92	0.86	5.53	6.88
(%)	(0.03)	(0.07)	(0.08)	(0.06)	(0.05)	(0.04)	(0.027)	(<0.001)
C:N	64.18	63.70	60.40	51.09	57.93	63.02	4.03	22.94
	(2.62)	(5.61)	(5.86)	(3.56)	(3.44)	(2.33)	(0.056)	(<0.001)

664 Sapwood: control, *n* = 10; defoliated, *n* = 8. Needles: *n* = 3.

665 Abbreviations: 0-yr = current-year needles (from 2009), 1-yr = one-year-old needles (from 2008), 2-yr =
 666 two-year-old needles (from 2007), Treat. = Treatment.

667

668 **Table 3.** Radial, height and tree-ring width growth of control and defoliated *P. nigra* trees included in the
 669 long-term PPM defoliation experiment along with the results of statistical tests (GLMs) on treatment
 670 effects. GLMs (Least Squares) were fit with “treatment” (control/ defoliated) as a fixed factor and the size
 671 of trees at the beginning of the experiment (DBH and height of trees in 1992) as covariates. Treatments
 672 were applied between 1996-2003 (treatment period) while trees were left to recover between 2004-2009
 673 (recovery period). Trees were finally harvested in 2009, when the experiment terminated. Growth data are
 674 shown for the last year of the treatment period (2003, “end treatment period”) and also as the average
 675 across treatment and recovery periods. Significant effects ($P < 0.05$) are shown in bold, *d.f.* = 1 in all
 676 cases.. See materials and methods for further details on the design and analysis.

Variables	Control		Defoliated		Statistics	
	Mean	SE	Mean	SE	<i>F</i>	<i>P</i> -value
Radial growth (cm)						
DBH end treatment period	14.4	0.7	9.4	1.1	48.01	<0.001
Treatment period	6.8	0.7	0.9	0.2	48.01	<0.001
Recovery period	2.2	0.2	1.3	0.3	7.95	0.014
DBH final harvest	16.5	0.8	10.8	1.3	39.23	<0.001
Height growth (m)						
End treatment period	0.4	0.02	0.02	0.004	145.38	<0.001
Treatment period	2.8	0.2	0.4	0.05	98.71	<0.001
Recovery period	1.5	0.1	0.6	0.1	59.76	<0.001
Final harvest	0.4	0.02	0.3	0.05	5.47	0.035
Tree-ring width (mm)						
End treatment period	3.2	0.4	0.03	0.02	24.46	<0.001
Treatment period	2.3	0.1	0.2	0.04	30.47	<0.001
Recovery period	2.2	0.1	1.2	0.1	9.44	0.009
Final harvest	1.8	0.2	1.5	0.2	0.92	0.211

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679 **Table 4.** Input variables for non-structural carbohydrates (NSC) and growth included in the different data
 680 sets used to construct the structural equation models evaluated and summary of statistical indices
 681 indicating their goodness of fit. All models conformed to the same theoretical model shown in Fig 4.
 682 RMSEA index rendered values lower than 0.001 for all models. The best model for each index is
 683 highlighted in bold. Overall, data set 6 is the one that produced the model with the best fit.

Data set	NSC*	Growth†	Chi-square‡	P	RMR	GFI	AGFI	NFI	AIC
1	Needle	Needle BM	0.326	0.850	0.190	0.997	0.980	0.998	26.326
2	Needle	Branch Len.	0.412	0.814	0.281	0.997	0.975	0.997	26.412
3	Needle	Ring width	0.268	0.875	0.083	0.998	0.984	0.998	26.268
4	Stem	Needle BM	0.364	0.833	0.077	0.997	0.978	0.995	26.364
5	Stem	Branch Len.	0.924	0.630	0.136	0.992	0.944	0.988	26.924
6	Stem	Ring width	0.151	0.927	0.017	0.999	0.991	0.997	26.151

684

685 * Indicates origin of the NSC data included in the models.

686 † Abbreviations: Needle BM = Current-year needle biomass production; Branch Len. = Branch length

687 growth; Ring width = total current-year tree- ring width.

688 ‡ All chi-squares were not significant at $\alpha = 0.05$.

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691 **Table 5.** Input variables and standardized path coefficients (*P* values, in parentheses) of the different
 692 predictor → predict and relationships included in the structural equation models (*n* = 50 in all cases) built
 693 with varying combinations of the different data sets available (see input variables). Note these models
 694 were not considered in the main SEM analysis represented in Fig. 4, since they showed a poorer fit than
 695 the selected model number 6 (see Table 4 for indexes of goodness of fit). All models conformed to the
 696 same theoretical model shown in Fig 4. Significant effects (at $\alpha = 0.05$) are indicated in bold.

	Data 1	Data 2	Data 3	Data 4	Data 5
Input variables					
NSC*	Needles	Needles	Needles	Sapwood	Sapwood
Growth†	Needle BM	Branch Length	Ring width	Needle BM	Branch Length
Relationships					
Defoliation → NSC (April)	-0.699 (<0.001)	-.699 (<0.001)	-.699 (<0.001)	-0.340 (0.011)	-0.340 (0.011)
Defoliation → N (April)	0.396 (0.003)	0.396 (0.003)	0.396 (0.003)	0.396 (0.003)	0.396 (0.003)
NSC (April) → Growth	0.009 (0.959)	0.017 (0.917)	0.023 (0.906)	-0.187 (0.135)	-0.211 (0.089)
Defoliation → Growth	0.286 (0.104)	0.333 (0.058)	0.043 (0.832)	0.221 (0.102)	0.254 (0.058)
N (April) → Growth	0.366 (0.005)	0.329 (0.012)	0.203 (0.182)	0.357 (0.005)	0.319 (0.012)
Growth → NSC (Sept. - April)	-0.104 (0.079)	-0.117 (.045)	-0.048 (0.370)	-0.171 (0.139)	-0.303 (0.006)
NSC (April) → NSC (Sept. - April)	-0.907 (<0.001)	-0.912 (<0.001)	-0.890 (<0.001)	-0.763 (<0.001)	-0.806 (<0.001)
N (April) → NSC (Sept. - April)	0.365 (0.005)	0.167 (0.004)	0.130 (0.019)	-0.016 (0.887)	0.033 (0.751)

697

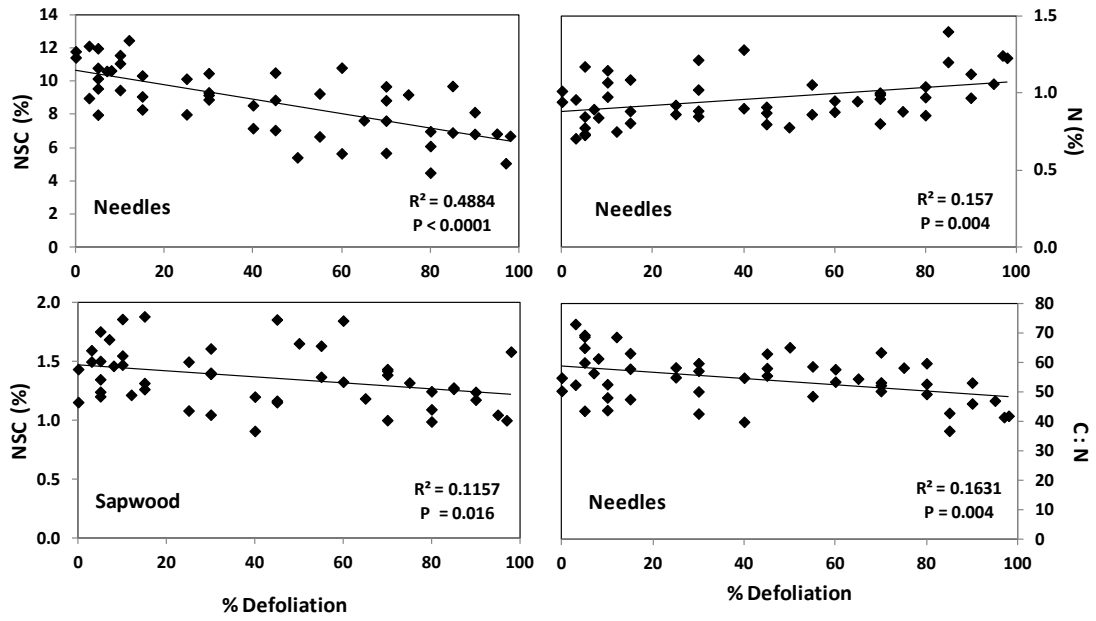
698 Abbreviations: NSC (April) and NSC (Sept. - April): non-structural carbohydrate concentrations
 699 measured in April and difference between April and September 2011 NSC concentrations, respectively; N
 700 (April): nitrogen concentrations measured in April 2011.

701 * Indicates origin of the NSC data included in the models.

702 † Abbreviations as in Table 4.

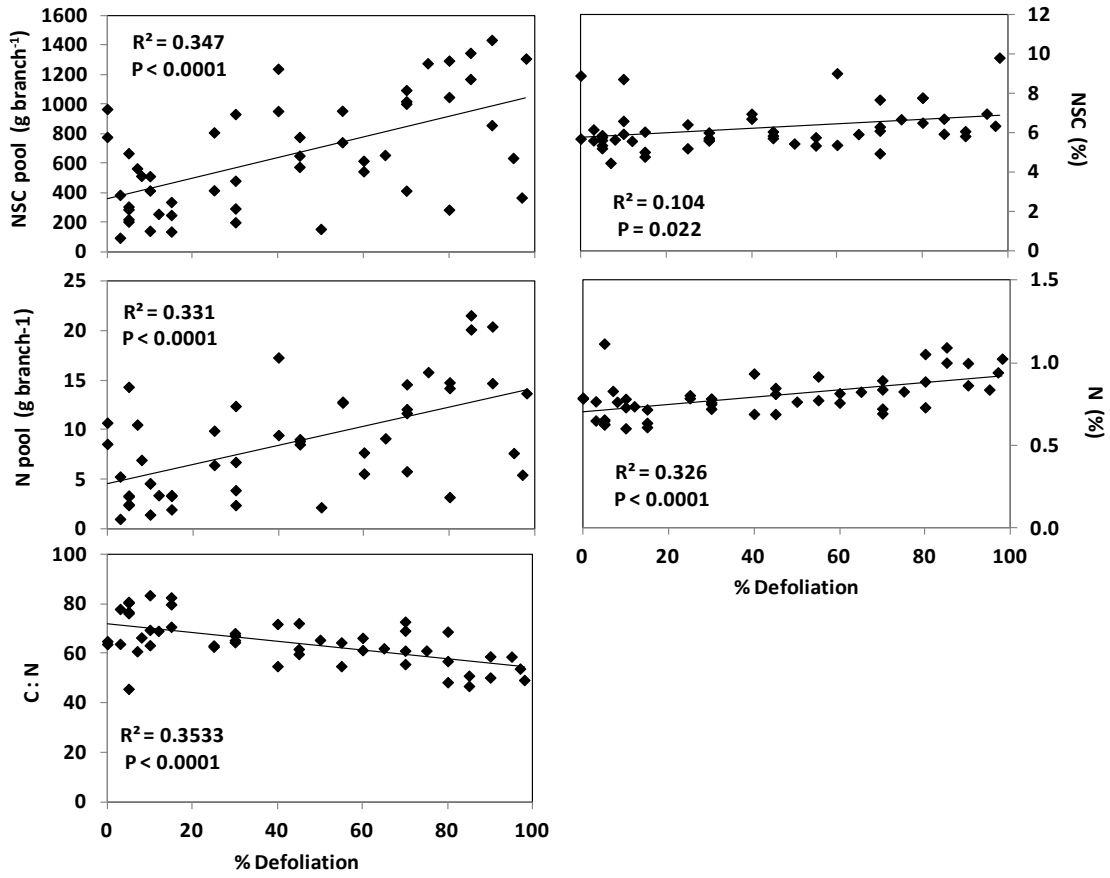
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705 **Figure 1**



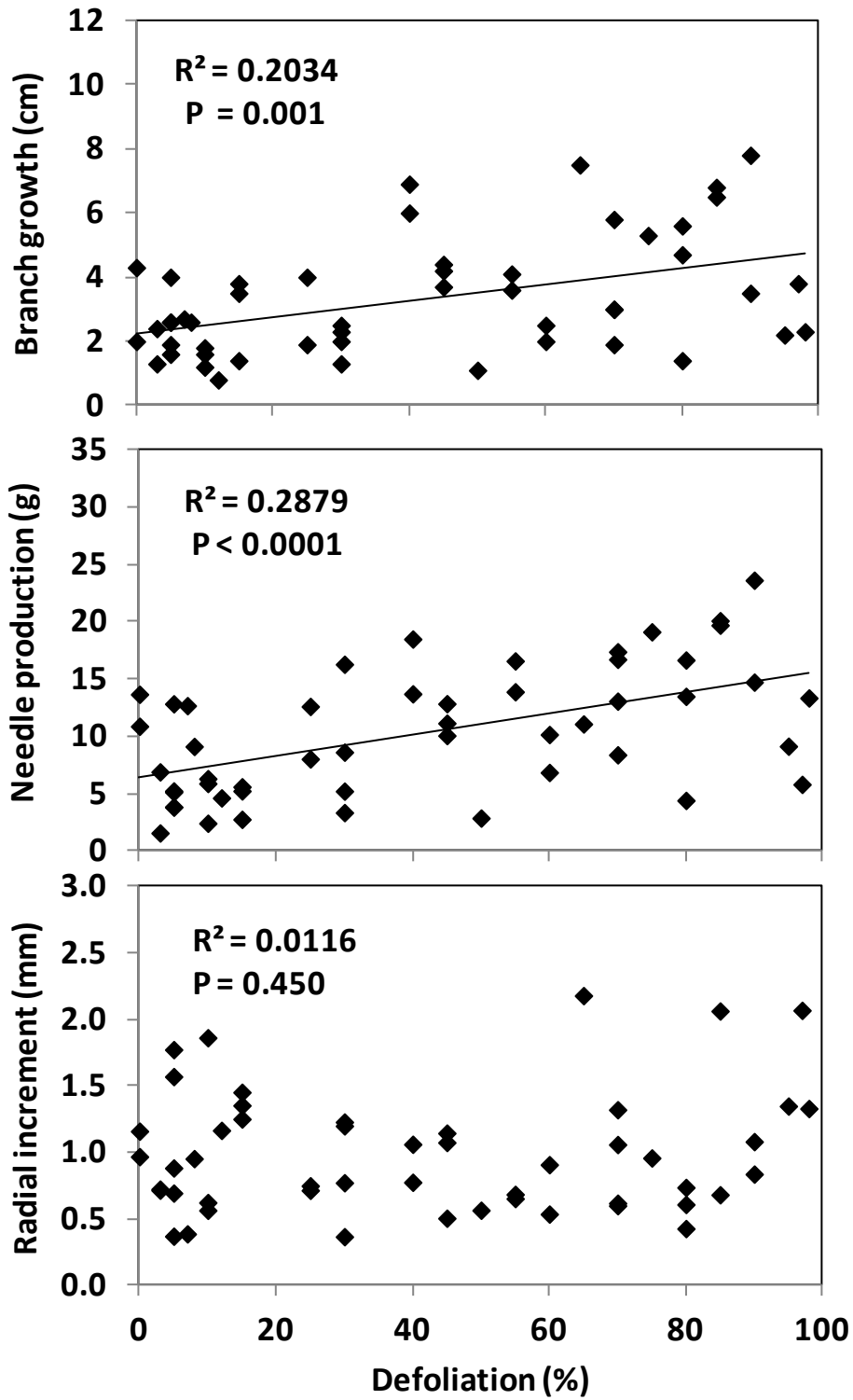
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708 **Figure 2**



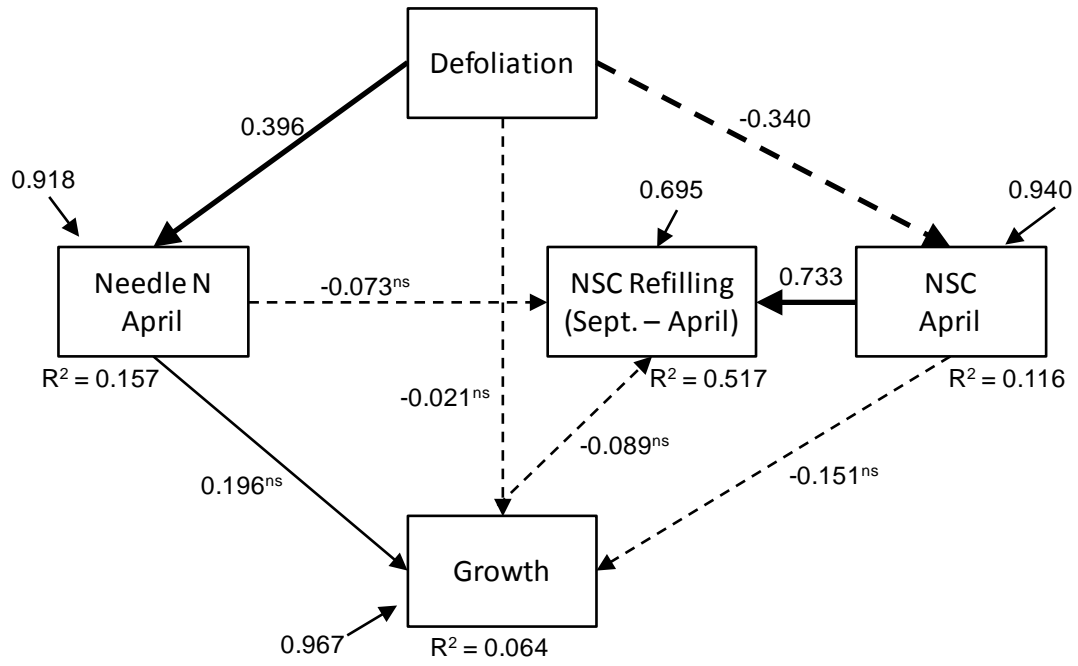
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711 **Figure 3.**



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713 **Figure 4.**



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