

1 **Growth and nutritional response of *Pinus pinaster* after a large pine weevil (*Hylobius***
2 ***abietis*) attack**

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4 Luis Sampedro^{a,1,*}, Xoaquín Moreira^{a,1}, Patricia Martínez^a and Rafael Zas^b

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6 ^a Centro de Investigación Ambiental CINAM-Lourizán, Apdo. 127, E-36080 Pontevedra,

7 Spain.

8 ^b Misión Biológica de Galicia (CSIC), Apdo. 28, E-36080 Pontevedra, Spain.

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17 *Corresponding author: lsampe@uvigo.es

18 Phone Number: +34986805078

19 Fax: +34986856420

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21 ¹ L.S. and X.M. contributed equally to this work

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25 **Abstract**

26 *Hylobius abietis* is an important pest of coniferous plantations in Europe, to which high
27 mortality, stem deformities and growth loss are typically attributed. In pine trees, as in other
28 long-lived organisms, the costs of short term resistance against invading organisms could be
29 shown in the long term. We examined the nutritional status of *Pinus pinaster* after a two-
30 years long *H. abietis* attack, measuring needle and phloem N and P concentrations, and the
31 impact of the damage on subsequent growth, survival and stem deformities over a period of
32 five years. The study sites were a *P. pinaster* family x fertilization trial, and a neighbouring
33 twin trial not attacked with similar climate and soil characteristics. Growth losses after the *H.*
34 *abietis* attack were important (up to 40%) but restricted to the first years after the attack. Five
35 years after the attack the annual height increment of pines in the attacked stand was not
36 related to the initial damage suffered, and plants showed regular stems, normal leader
37 dominance and regular height after 5 yr. These findings, consistent with the comparison
38 between stands, suggest a strong compensatory growth and that *P. pinaster* is a tolerant
39 species to the large pine weevil. Needle nutrient concentrations in the healthy stand were, as
40 expected, significantly greater in experimentally fertilized plants, and they were linearly
41 related to those in phloem showing equilibrated stoichiometry both for nitrogen ($r = 0.86$; p
42 <0.01 ; $N = 25$) and phosphorus ($r = 0.84$; $p <0.01$; $N = 25$). However, at the attacked stand,
43 nutrient concentrations in the needles did not follow the experimentally manipulated nutrient
44 availability in soils; and phosphorus concentration in the needles was unexpectedly not
45 related to those in the phloem. The pine seedlings attacked by *H. abietis* showed altered
46 potential of allocating nutrients to their tissues according to the nutrient availability existing
47 in the soil, and also altered stoichiometry in N and P concentration among phloem and
48 leaves. Maritime pine seem to be tolerant to the pine weevil attack, at least in the conditions
49 of this study, where pine weevil damage caused a deep alteration of nutrient allocation and
50 nutritional status. Further research is needed to elucidate to what extent altered nutrient

51 allocation may be part of an induced response to the attack or just derived from the vascular
52 injury caused by the weevil wounding in the phloem.

53

54 *Keywords:* Herbivory · Fertilization · Forest Pest · Resistance · Tolerance · Nutrient
55 allocation

56

57 **Introduction**

58 Damage by the large pine weevil, *Hylobius abietis* L. (Coleoptera: Curculionidae) is
59 the most severe threat to newly planted conifer seedlings after clearcutting in Europe
60 (Nordlander et al. 2003). Adults feed on the stem bark and phloem of young seedlings,
61 causing great economic losses if no protection measures are applied (Orlander and
62 Nordlander 2003; Petersson and Orlander 2003; Nordlander et al. 2008). The pine weevil has
63 been estimated to cause the death of up to 80% of coniferous seedlings planted following
64 clear-cutting (von Sydow and Birgersson 1997; Orlander and Nilsson 1999; Nordlander et al.
65 2008), and severe growth losses in the surviving seedlings (Orlander and Nilsson 1999; Zas
66 et al. 2006). Besides mortality and growth reductions, *H. abietis* attack can cause the leader
67 loss by stem girdling, which can lead to stem form defects in those plants where some lateral
68 branches emerge to recover apical dominance. Although there is a clear consensus that *H.*
69 *abietis* cause important growth reduction and stem deformities, experimental data supporting
70 those effects are scarce. Furthermore, most of the papers reporting growth losses are usually
71 limited to one or two years after damage.

72 Besides growth loss and stem deformities, the nutritional status and nutrient
73 allocation of plants could also be influenced by insect grazing. Plants subjected to insect
74 attack have been found to present greater or lower nutrient concentrations in their plant
75 tissues, and even altered within plant variability in nutrient concentrations, than their
76 uninfested counterparts (Polley and Detling 1989; Ayres et al. 2000). In a revealing

77 experimental study, Newingham et al. (2007) recently reported a significant shift in nitrogen
78 allocation to the shoot in the flowering plant *Centaurea maculosa* (Asteraceae) when infested
79 with a belowground herbivore. However, little is known about the existence of herbivore
80 induced responses involving altered resource allocation patterns in conifers or other long-
81 lived plants.

82 Most of *H. abietis* damage has been reported in spruce and Scots pine (Orlander and
83 Nilsson 1999; Orlander et al. 2000), but *Pinus radiata* and other European southern pines
84 such as maritime pine (*Pinus pinaster* Ait.) are also a target species of this generalist phloem
85 herbivore. Maritime pine is the most important forest tree species in Galicia (NW Spain),
86 occupying nearly 400,000 ha (~27% of the Galician wooded area). In 2003 several *P.*
87 *pinaster* family × fertilization trials were established to test the plasticity of 28 half sib
88 families of the actual Galician maritime pine breeding population in relation to soil fertility,
89 one of the main factors determining site index of coniferous stands in Galicia (e.g. Sánchez-
90 Rodríguez et al. 2002). One of those trials suffered a massive attack of *H. abietis* just after
91 planting. We studied the differential preference of the weevil due to the fertilization and the
92 genetic material in two companion papers (Zas et al. 2005; 2006). Briefly, we found that
93 wounding intensity by the pine weevil in fertilized trees was up to 2.9-fold greater than in
94 unfertilized control plants during the two consecutive years of attack; families markedly
95 differed in their susceptibility to the insect; and damage by the weevil produced deep impact
96 on fitness, where mortality was closely correlated to wounding intensity, and more damaged
97 plants showed reduced growth rates in the following growing season.

98 In this paper we describe how two consecutive years of large pine weevil attack in
99 this *P. pinaster* family × fertilization trial affected the nutrient allocation and the subsequent
100 growth recovery in the surviving seedlings. We studied the nutrient concentration in needles
101 and phloem immediately after the attack, and we measured the plant growth during five years
102 after planting. As we lack reference nutrient values from the seedlings before the attack,

103 because weevil damage began too early after planting, we also studied the same properties in
104 a neighbouring twin family × fertilization trial free of *H. abietis* damage with similar climate
105 and soil properties, and identical design and genetic material.

106

107 **Material and methods**

108 **Location and description of the genetic trials**

109 We studied two twin *P. pinaster* experimental field trials located in the west coast of
110 Galicia (NW Spain) separated by 30 km. The trial attacked by the pine weevil, previously
111 described in companion papers (Zas et al. 2005; 2006), is located at Rianxo (42.36° N; 8.46°
112 W; altitude 90 m a.s.l.; 6 km from the sea). The healthy stand used as reference is located at
113 Rebordelo (42.46° N; 8.48° W; altitude 530 m a.s.l.; 18 km from sea). The climate in both
114 sites is temperate humid Atlantic. Annual precipitation during the study period (2003 – 2007)
115 was 1511±195 mm and 1976±221 mm, and the mean annual temperatures were 14.8±1.9 °C
116 and 12.1±0.6 °C in Rebordelo and Rianxo, respectively. Monthly precipitation and mean
117 monthly temperature strongly correlated between the two sites during those years ($R^2 = 0.900$
118 and $R^2 = 0.989$ respectively, $N = 60$, $p < 0.001$).

119 Soils in both sites derived from granite and they are thin, sandy and acidic. Main
120 characteristics just before plantation were low pH (pH in H₂O Rebordelo ~ 4.0; Rianxo ~
121 4.3), high organic matter content (~180 and 170 g kg⁻¹ soil ash-free dry weight in Rebordelo
122 and Rianxo, respectively), high total nitrogen content (Rebordelo ~ 7.1 g N kg⁻¹ soil; Rianxo
123 ~ 6.3 g N kg⁻¹ soil), and very low concentrations of other nutrients, especially of available
124 phosphorus (Rebordelo Olsen P ~ 4.2 mg kg⁻¹ soil; Rianxo Olsen P ~ 4.7 mg kg⁻¹ soil).
125 Before trial establishment, Rebordelo stand was covered mainly by *Ulex europaeus* L., and
126 Rianxo stand was covered by a 35 year-old *P. pinaster* stand. The clear-felling of the latter
127 stand in January 2002 motivated the subsequent weevil infestation.

128 The experimental design was identical in both family × fertilization trials. It was a
129 split-plot block design replicated in 10 blocks, with the fertilization treatments acting as the
130 main factor, and the pine families as the split factor. Whole plots were around 12 x 18 m in
131 size. In total, we planted 2790 seedlings in each trial, corresponding to 10 blocks × 9
132 fertilization treatments × 31 pine genetic entries. A more detailed description of the trials is
133 in Zas et al. (2006).

134 Six-month-old containerized *P. pinaster* seedlings were planted at beginning of
135 March 2003. Immediately after planting, nine fertilization treatments (with different
136 combinations of four commercial fertilizers) were randomly assigned to the whole plots
137 within each block, and manually distributed 30 cm around the seedlings. Plant material in
138 both trials consisted of 28 open-pollinated families from plus trees randomly selected in a
139 first generation seed orchard (Sergude, 42.82° N, 8.45° W), and three commercial seed
140 sources as controls. Seedlings were grown together at the same nursery for both sites, and
141 were about 15 cm tall at planting. Seedlings at Rianxo were attacked by *H. abietis* during 18
142 months, since 3 months after planting (June 2003) until the end of autumn of the second year
143 (December 2004), with peaks of damage at late spring and early autumn. The spatial analysis
144 of the infestation revealed that the damage was uniformly spread throughout the experimental
145 area (Zas et al. 2006).

146

147 **Sampling and field assessments**

148 In order to reduce the analytical effort to reasonable levels, for the purpose of the
149 present paper we studied a randomly selected subsample of the whole trials at each site. We
150 sampled eight families under five fertilization treatments in five blocks; and additionally
151 three of these families were sampled in all the 10 blocks of the trials in order to account for
152 the spatial variability. The sample size was 275 trees for each trial.

153 The four fertilization treatments selected for tree sampling within the trials had a
154 common base of potassium sulphate (applied at 15 g K plant⁻¹) and magnesium sulphate (at 5
155 g Mg plant⁻¹), plus an alternative combination of presence or absence of ammonium nitrate (5
156 g N plant⁻¹) and calcium phosphate (10 g P plant⁻¹). In summary, four fertilization treatments
157 (+N+PCa; -N+PCa; +N-PCa; -N-PCa) plus an unfertilized control were selected for this
158 study.

159 Plant growth was measured yearly (well in November or December) from 2003 to
160 2007 in both trials. Growth variables measured were total height in cm and stem-base
161 diameter in mm. Stem deformities (bottom stem bifurcations and strong bottom stem
162 curvatures) were evaluated in the fifth year as a binomial variable (presence-absence) in both
163 trials.

164 The damage by *H. abietis* in the attacked stand was assessed during the first
165 (February 2004) and the second year (December 2004) after planting. The intensity of
166 wounding was evaluated by estimating the relative debarked area along the stem caused by
167 the weevil using a four-level scale (0 = undamaged, 1 = some wounds, 2 = many wounds,
168 and 3 = death due to girdling). To minimize subjectivity, the pine stem was divided in ten
169 parts using an elastic ruler, recording the weevil wounding score in each part, and summing
170 the ten values up, resulting in a 0-30 scoring for each tree. For the second year evaluation we
171 followed the same method and scale of damage, but the stem height was only divided in five
172 parts. As wounding preferences were extensively studied in a former paper for the whole trial
173 (N = 2790 trees; Zas et al. 2006), for exploring the relationship with the nutritional status and
174 subsequent growth increments in the present study we exclusively considered the wounding
175 score in the 275-trees subsample.

176 The nutritional status was studied two years after planting (December 2004),
177 immediately after the insect attack at Rianxo. We evaluated nitrogen and phosphorus
178 concentration in the needles of all the 275 plants of each trial. In each tree, a group of about

179 100 needles from the apical stem and orientated towards the West was collected in December
180 2004, when nutrient concentrations are relatively stable (Will 1971), and transported to the
181 lab in ice coolers. Needle samples were oven dried at 65 °C to constant weight, finely
182 ground, labelled and preserved for chemical analysis.

183 To study the nutrient allocation, 25 seedlings were destructively sampled in the same
184 five fertilization treatments, and in five randomly selected blocks in each site. These
185 seedlings belonged to the mixed commercial seedlot produced in the same seed orchard as
186 the studied families, which is formed by a random representation of the seeds produced by all
187 the 116 plus trees in this seed orchard. A section 15 cm long of the apical stem (2004 growth)
188 was sampled and transported in ice coolers to the lab, where the phloem was immediately
189 separated by hand using a surgical knife, oven-dried and processed as above. Needles of
190 these trees were sampled and processed as previously described.

191

192 **Chemical analysis**

193 For nitrogen and phosphorus content, 0.3 g of phloem and needles were digested in a
194 mixture of selenous sulphuric acid and hydrogen peroxide (Walinga et al. 1995). Nitrogen
195 was colorimetrically analysed in diluted aliquots of this digestion using a BioRad 680
196 microplate reader (California, USA) at $\lambda = 650$ nm (Sims et al. 1995). Phosphorus was
197 analysed in the same diluted aliquots by inductively coupled plasma optical emission
198 spectroscopy (ICP-OES) using a Perkin-Elmer Optima 4300DV (Massachusetts, USA) in the
199 central laboratory facilities at Universidade de Vigo – CACTI
200 (www.uvigo.es/webs/cactiweb/). Nitrogen and phosphorus concentration were expressed in
201 mg g^{-1} dried weight of tissue. Sample size for foliar nutrient content was $N = 275$ in each
202 stand; and for phloem-needle relationships $N = 25$ in each stand.

203

204 **Statistical analyses**

205 Within each site, nutrient contents and final tree growth were analyzed using the
206 PROC-MIXED procedure of the SAS System (Littell et al. 2006) and the following within
207 site mixed model $Y_{ijk} = \mu + Fer_i + Fam_j + B_k + Fer \times Fam_{ij} + Fer \times B_{ik} + \varepsilon_{ijk}$, where μ is the
208 general mean, Fer_i , Fam_j and B_k are the main effects of fertilization treatment i ($i = 1$ to 5),
209 family j ($j = 1$ to 8) and block k ($k = 1$ to 10), $Fer \times Fam_{ij}$ and $Fer \times B_{ik}$ are the
210 corresponding interactions, and ε_{ijk} is the experimental error. To analyze the whole plot factor
211 (i.e. fertilization) with the appropriate error term, the $Fer \times B$ interaction was considered a
212 random effect (Littell et al. 2006). For the purpose of the present paper, the main factor of
213 study was fertilization and their interactions, and we do not present results regarding genetic
214 effects. Family, as block, was anyway included in the model for reducing the residual
215 variance to improve the power of the analyses. The genetic variation in pine susceptibility to
216 the insect for the first and second year assessments were extensively studied in a companion
217 paper (Zas et al., 2005). When main effects were significant, differences among treatment
218 means were tested for significance using the LSMEAN statement (SAS-Institute 1999). Data
219 are shown as least square means \pm standard error (LS means \pm s.e.).

220 To compare height and diameter growth over the five years between the two test sites
221 we performed a repeated measures mixed model across sites using the PROC-MIXED
222 procedure of the SAS System (Littell et al. 2006). The model included site (main factor),
223 block (nested in site), fertilization (whole plot factor), family (split factor), time (repeated
224 measures), and the corresponding interactions. A first order autoregressive model was
225 assumed for the covariance structure of the repeated measures. For adequately account for
226 the multisite split-plot design, the model included four different error terms.

227 Differences in binomial variables between stands were analyzed with a chi-square
228 test. Linear regressions and Pearson correlations were used to evaluate the relationships
229 between weevil damage, pine growth and nutrient contents in plant tissues. These analyses
230 were carried out on family means ($N = 8$) and on raw data ($N = 275$ for the subsample of

231 families used in foliar nutrient status; and $N = 25$ for the destructive sample of the
232 commercial seedlot used in needle-phloem correlations).

233

234 **Results**

235 **Growth loss**

236 Repeated measures analysis of tree height performed on the 5 years series in the two
237 sites revealed a significant site \times time interaction ($F_{4,1847} = 67.5$, $p < 0.001$). No significant
238 differences between the attacked and the not attacked stands were found in height of the
239 sampled trees at the first year after planting (Fig. 1a), suggesting similar forest site-quality
240 for both stands. The intense pine weevil attack promoted significant growth losses during the
241 following years in the infested stand, as revealed by a growth reduction of 40%, 15% and 5%
242 in the second, third and fourth year, respectively, comparing to the growth observed in the
243 stand with no attacks (Fig. 1a). However, plants in the attacked stand had apparently
244 compensated for earlier growth losses by the 5th year, and overcome in size those in the not
245 attacked stand.

246 Five years after the attack, the total height increment was not significantly related to
247 the intensity of damage suffered ($r = -0.10$, $p = 0.12$, $N = 275$).

248 A similar trend was observed for diameter growth, with a significant site \times time
249 interaction ($F_{2,1137} = 21.20$, $p < 0.001$; Fig. 1b). During the second and the third year, diameter
250 of plants in the infested stand was 35% and 25% lower than in the not attacked stand,
251 respectively (Fig. 1b). However, no significant differences between stands were observed in
252 diameter growth in the fifth year, indicating complete compensation of diameter growth, as
253 with height growth (Fig. 1b).

254 The early growth response to the fertilization treatments was significant in both trials
255 (Fertilization $F_{4,36} = 2.78$, $p < 0.05$ at Rianxo, and $F_{4,36} = 10.1$, $p < 0.001$ at Rebordele). The
256 observed responses to fertilization during the first year were similar between sites, where the

257 pine height produced by the best fertilizer treatments was 53 ± 3 cm at Rianxo and 61 ± 3 cm
258 at Rebordelo; while those achieved in unfertilized controls were respectively 48 ± 3 cm and
259 44 ± 2 cm. However, after five years, height of fertilized trees (overall mean height of
260 fertilized trees = 362 ± 3 cm) was significantly greater than the control (315 ± 3 cm) in the
261 healthy stand (Table 1; Fig. 2e), whereas the effect of fertilization on final height and
262 diameter at age five was lost in the attacked stand (Table 1; Fig 2f).

263

264 **Mortality and stem deformities**

265 Although 94% of the seedlings in the attacked stand (Rianxo) were damaged by the
266 insect, mortality was only 12% after one year and 17% after two years (% of planted
267 seedlings). These values were only slightly higher than the mortality registered in the healthy
268 stand (3% and 8% after the first and second year, respectively).

269 In the infested stand, damage by the pine weevil caused the leader loss of 35% and
270 48% of the seedlings one and two years after the attack, respectively (Zas et al., 2006).
271 However, although bottom stem deformities were significantly more frequent in the infested
272 stand ($\chi^2_1 = 17.3$, $p = 0.0003$), only 4.6% of the surviving seedlings showed severe stem
273 deformities five years after the attack, indicating a good recovery of apical dominance by
274 lateral branches.

275

276 **Nutritional status**

277 Increasing experimentally the nutrient availability in the not attacked stand
278 significantly affected, as expected, the phosphorus and nitrogen concentration in the needles
279 (Table 1, Fig. 2a, 2c). Phosphorus needle concentration was significantly greater in the
280 treatments with phosphorus addition than in the unfertilized control (Fig. 2a). Nitrogen
281 content, which is considered the superfluous nutrient in Galician soils, was generally greater
282 in the unfertilized control than in the fertilized plants (Fig. 2c). Conversely, no significant

283 effect of the fertilization treatments was detected on needle nutrient concentrations in the
284 attacked stand (Table 1; Fig. 2b, 2d).

285 Phloem and needle nutrient concentrations showed a strong and positive linear
286 relationship in the not attacked stand for nitrogen (Fig. 3a) and phosphorus (Fig. 3b).
287 However, an unexpected lack of relationship was observed for phloem-needle phosphorus in
288 the infested stand (Fig. 3b), while only a weak relationship appeared for nitrogen
289 concentration between those tissues (Fig. 3a), evidencing altered stoichiometry in plant
290 tissues.

291 Wound intensity in the second year, i.e. just before needle sampling, was significantly
292 and negatively correlated with N and P needle concentration when analysed on a raw data
293 basis ($r = -0.28$; $N = 275$; $p < 0.001$ for N, and $r = -0.13$, $N = 275$, $p < 0.05$ for P), and with P
294 concentration when analysed on a family mean basis ($r = -0.64$, $N = 8$, $p < 0.05$).

295

296 **Discussion**

297 **Impact of weevil attack on pine growth, stem deformities and mortality**

298 The large pine weevil, *H. abietis*, is an important insect pest of coniferous
299 reforestation in Europe, to which important growth losses are commonly attributed. Our
300 results showed a significant plant growth reduction in the infested stand during the following
301 three years after the attack. During those years, plants showed an abnormally low interannual
302 growth rate that suggests an important energy investment in repairing the damage suffered.
303 Growth losses due to attacks by other weevil species have been previously reported, but little
304 information is available in the case of *H. abietis*. White pine weevil (*Pissodes strobi*) attack
305 reduced *Pinus strobus* height growth by 40 to 60% in a year (Hamid et al., 1995), and
306 reduced significantly the total height in spruce plantations 10 years after planting (Kiss and
307 Yanchuk 1991; King et al., 1997). Spruce growth remained negatively correlated with the
308 initial *P. strobi* damage up to six years after the attack (vanAkker et al., 2004). However, *P.*

309 *pinaster* seedlings in our study were able to compensate for their early growth reduction,
310 both in height and diameter, by the fifth year. To our knowledge this is the first work
311 reporting subsequent growth patterns suggesting compensatory growth in a conifer plantation
312 attacked by *H. abietis*. Plant growth compensation for insect damage has been commonly
313 reported in long-lived woody plants (Trumble et al. 1993; Edenius et al. 1993; Bast and
314 Reader 2003), and can be interpreted as a tolerance mechanism by which trees can reduce the
315 impact of herbivores in plant fitness (Strauss and Agrawal 1999), allocating more resources
316 to vegetative growth than undamaged trees. In this sense, *P. pinaster* seems to be highly
317 tolerant to *H. abietis* at least on these sites.

318 The inference of compensatory growth was strongly supported by the fact that pine
319 height growth in the attacked stand was unrelated to initial weevil damage five years after the
320 infestation, while that relationship was strongly negative (the most wounding the less
321 subsequent annual growth) in 2 and 3 yrs old pine seedlings (Zas et al., 2006). Thus, pines
322 were able to overcome the initial growth reductions caused by the insect.

323 The similar pine heights at the 5th year in the attacked and in the not attacked stand
324 also support this compensatory growth investment. However, as in other “natural
325 experiments” this conclusion relies on the assumption that site quality was similar in both
326 sites, and that the two sites would have produced equivalent growth in the absence of insect
327 attacks. We assumed same site quality based on (i) the similar soil properties before planting;
328 (ii) similar climate, based on last 20 yrs precipitation and temperature mean values, the
329 specific values observed during the experiment, and the close correlations between both sites
330 for precipitation and temperature regimes (see Methods section); (iii), the same early growth
331 response to the fertilization treatments; (iv) the same early growth of unfertilized seedlings in
332 both stands; and our personal experience with this species in the area. However, we cannot
333 ensure exactly the same forest-site index for both stands, neither the absence of unaccounted
334 site effects. Thus, the observed growth patterns could be alternatively explained simply by

335 better site quality in the attacked stand, promoting greater annual growth rates after the insect
336 attack. Further manipulative experiments with protected control plants would definitely
337 demonstrate the compensatory growth investment suggested by the comparison between
338 stands.

339 The intensity of the attack was fairly high in the studied trial, with near all plants
340 attacked at some level, and near half of the seedlings losing their leaders. However, five
341 years after the attack the surviving trees showed very few stem deformities (less than 4% of
342 trees), and a generalized recovering of the apical dominance. These results also suggest high
343 tolerance of *P. pinaster* to the pine weevil in the study area.

344 In this study we observed much lower mortality due to *H. abietis* than those reported
345 for northern European coniferous forests, where mortality commonly reach up to 80% of the
346 seedlings planted following a clear-cutting (von Sydow and Birgersson 1997; Orlander and
347 Nilsson 1999). Some hypotheses could contribute to explain these findings. In one hand, the
348 suggested tolerance of this pine species, discussed above, could favour a lower mortality with
349 the same level of damage. In agreement with this idea, Zas et al. (2008) found that mortality
350 of *Pinus radiata* seedlings due to weevil damage was nearly twice that observed in *P.*
351 *pinaster* adjacently planted for the same lever of insect damage (Zas et al. 2008). On the
352 other hand, a lower mortality of *P. pinaster* after *H. abietis* attack could simply result from a
353 lower intensity of damage to each individual tree. Pine weevil populations in higher latitudes
354 seem to be denser than those observed in the study area (Moreira et al., 2008). Besides,
355 weevil biology could be constrained by biogeographical considerations, because the studied
356 area represents its southern limit of the distribution (Orlander and Nilsson 1999).
357 Furthermore, reduced availability of oviposition sites in Galicia could lead to weaker booms
358 of emerging insects. The pine plantations after clear-cutting in NW Spain are clearly fewer
359 and smaller than those observed in northern forests. Unfavourable conditions for the
360 aggregation of *H. abietis* after clear-cutting are also probable because Galician landscape is

361 fragmented and composed by a mosaic of small size properties of broadleaves and coniferous
362 stands, interspersed with grasslands, cropland and scrub, lacking of large continuous
363 extensions of coniferous forest.

364 In summary, all these findings consistently support that *P. pinaster* is a highly tolerant
365 species to this pine weevil, at least in the area of this study. Lombardero et al. (2008) have
366 also found evidences of high tolerance of *P. pinaster* to other insect herbivores at the same
367 area. These authors reported that the impact of bark beetle attacks on the growth of *P.*
368 *pinaster* was much lower than on other pine species, although the intensity of insect attacks
369 were more than twice greater in *P. pinaster*. In agree with these observations, reduced costs
370 of plant tissue reconstruction after herbivore grazing in favourable environments for high
371 primary production (as the warm temperature and high precipitation in the NW Spain), could
372 favour tolerance mechanisms against herbivory instead of investments in resistance (Strauss
373 and Agrawal, 1999; Fine et al., 2004).

374

375 **Impact on nutritional status**

376 The response to fertilization treatments in the healthy stand agrees with the common
377 deficiencies typically found in Galician forest soils. In this region P is a clear limiting
378 nutrient while N is well supplied or even in excess (Sánchez-Rodríguez et al. 2002; Zas and
379 Serrada 2003). And so, the regular trend observed in pine trees in these soils is an increase of
380 P needle concentration due to P fertilization; and a lack of a response to N additions (e.g. Zas
381 2003). The higher N content in the unfertilized controls can be explained by a dilution effect
382 in the fertilized trees because of their higher growth promoted by the base fertilization of
383 potassium and magnesium sulphate. However, and despite the early response in growth to the
384 fertilization treatments, we did not detect significant effects of fertilization on needle
385 phosphorus and nitrogen concentration in the attacked stand. In concordance with this
386 findings, lower concentrations in needle nutrient appeared inversely related to the previous

387 level of damage suffered. These results suggest that the attack by the pine weevil strongly
388 modified the potential of the pine seedlings to allocate their resources to the needles
389 according to the nutrient availability existing in the soil.

390 Another main finding of this study is that the seedlings attacked by *H. abietis* showed
391 altered stoichiometry in N and P concentration among phloem and needles. Strong positive
392 correlations among needles, stem, and root nutrient contents are commonly found in forest
393 plantations, and are the regular trend in coniferous seedlings (Parks et al. 2000). We found
394 strong positive relationships between the content of nutrients in phloem and needles in the
395 not attacked stand. Nutrient analysis of greenhouse-grown *P. pinaster* seedlings of the same
396 families also revealed a strong correspondence between nutrients in different tissues (Moreira
397 et al., unpublished data). Thus, the lack of the corresponding relationship in the infested stand
398 suggests that weevil damage is modifying the regular nutrient allocation patterns.

399 The results observed in the attacked stand are clearly anomalous and the most
400 plausible explanation for these anomalies is precisely the damage caused by the pine weevil,
401 which definitely characterize this site. Moreover, the fact that the relationship between
402 weevil damage and needle nutrient concentrations is significant and negative despite the
403 positive effect of fertilization on weevil damage also supports the hypothesis that the pine
404 weevil damage is the cause of the altered nutrient allocation pattern. However, due to the
405 experimental design, we cannot reject that site differences could also contribute to explain
406 the unexpected observed patterns.

407 It has been suggested that reduced or increased levels of nutrients in target plant
408 tissues could be part of induced responses to herbivory (Karban and Baldwin 1997;
409 Newingham et al. 2007). Furthermore, Newingham et al. (2007) documented not simply
410 altered nitrogen concentrations in target tissues of infested knapweeds but even allocation of
411 nitrogen away from the target tissues. Karban and Baldwin (1997) highlighted the
412 importance of such responses, not only because alterations of primary metabolites can

413 directly modify the tissue nutrient quality, but also because changes in primary metabolites
414 may also change the efficacy of secondary compounds.

415 The disruption of the normal nutrient allocation pattern following the insect attack
416 found in the present study could be part of this type of induced responses to herbivory. This
417 study would be the first time such a response is documented in pine trees. Alternatively to
418 this hypothesis, feeding on the phloem by the pine weevil may also directly interrupt the
419 nutrient transport along the stem due to the vascular injury caused by the weevil wounding
420 activity in the phloem. Manipulative experiments using chemical elicitors of induced
421 responses, such as methyl jasmonate, that does not involve physical damage in the vascular
422 tissues will definitively help to prove or reject the existence of induced changes in the
423 nutrient allocation patterns in long-lived plants such as pine trees. Disregarding whether the
424 altered nutrient allocation within plant tissues is part of an induced response mechanism to
425 the weevil attack, or it was derived directly from the vascular damage after insect wounding
426 on the phloem, our results indicate that plant nutritional status was largely altered by the
427 insect damage, which may have further important consequences on other plant vital
428 processes.

429

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512

513

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519 and INIA RTA07-100 projects.

520

521

522 Table 1. Summary of the mixed model for phosphorus (P) and (N) and concentration in the
 523 needles, and height in the fifth year (H₅) of *P. pinaster* seedlings at two twin family ×
 524 fertilization trials, one of them attacked by *H. abietis* (Rianxo) and another not attacked
 525 (Rebordelo).

526

527

| | ¹ DF _{num} | DF _{denom} | P | | N | | H ₅ | |
|--------------------------|--------------------------------|---------------------|------|--------------|------|--------------|----------------|--------------|
| | | | F | p>F | F | p>F | F | p>F |
| Rianxo (attacked) | | | | | | | | |
| Block | 9 | 36 | 2.23 | 0.043 | 0.74 | 0.668 | 0.50 | 0.867 |
| <u>Fertilization</u> | 4 | 36 | 1.41 | 0.251 | 0.96 | 0.441 | 0.80 | 0.531 |
| <u>Family</u> | 7 | 153 | 0.81 | 0.578 | 1.09 | 0.374 | 1.21 | 0.301 |
| Fam × Fert | 28 | 153 | 1.21 | 0.231 | 1.10 | 0.340 | 1.17 | 0.273 |
| Rebordelo (not attacked) | | | | | | | | |
| Block | 9 | 36 | 2.23 | 0.043 | 1.27 | 0.288 | 1.09 | 0.396 |
| <u>Fertilization</u> | 4 | 36 | 3.87 | 0.010 | 4.27 | 0.006 | 2.97 | 0.032 |
| <u>Family</u> | 7 | 177 | 2.38 | 0.024 | 2.29 | 0.029 | 0.87 | 0.528 |
| Fam × Fert | 28 | 177 | 0.75 | 0.813 | 0.75 | 0.810 | 0.96 | 0.531 |

528

529 ¹DF = degrees of freedom.

530

531 **FIGURE CAPTIONS**

532

533 Figure 1. Height (1a) and stem base diameter (1b) of *P. pinaster* seedlings during five
534 consecutive years after planting in two twin family × fertilization trials, one of them attacked
535 by *H. abietis* (Rianxo, black bars) and another not attacked (Rebordelo, white bars). Overall
536 LS means across all fertilization and treatments ± S.E. are presented, according to repeated
537 measures ANOVA. N = 275 per trial. Asterisks indicate significant differences at p <0.01
538 (**) and p <0.001 (***); n.s.: not significant.

539

540 Figure 2. Effects of experimental fertilization on the concentration of phosphorus (2a, 2b)
541 and nitrogen (2c, 2d) in the needles, and on the height at year 5 (2e, 2f) of *P. pinaster*
542 seedlings in two twin family × fertilization trials, one of them attacked by *H. abietis* (Rianxo,
543 right panels, black bars) and another not attacked (Rebordelo, left panels, white bars).
544 Samples for foliar analyses were taken immediately after the second year attack, two years
545 after planting. LS means ± S.E. N = 55 per treatment and trial. Different letters indicate
546 significant differences at p <0.05.

547

548 Figure 3. Nitrogen (a) and phosphorus (b) nutrient concentrations in needles vs phloem in 2
549 years-old *P. pinaster* seedlings in two twin family × fertilization trials, one of them attacked
550 by *H. abietis* (Rianxo, solid dots and lines) and another not attacked (Rebordelo, open dots,
551 dashed lines). Each point represents a randomly-selected destructively sampled tree
552 belonging to the same seed source at both stands. N = 25 for each site.

553

554

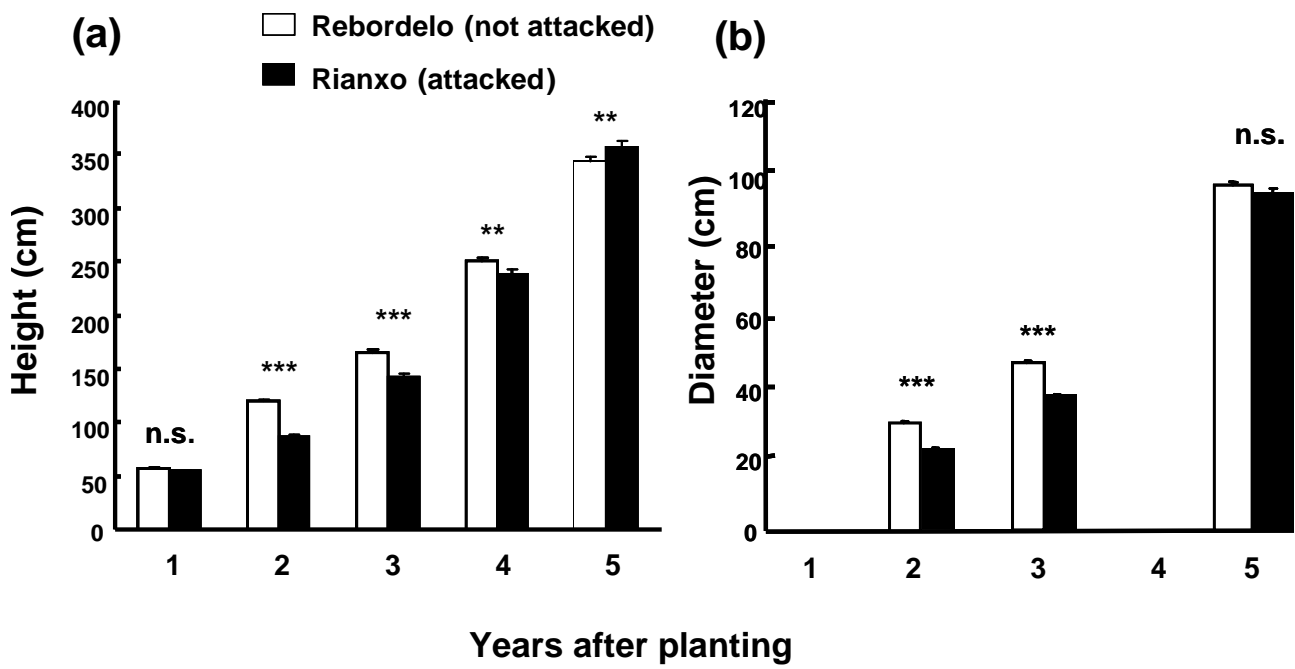


Figure 1. Sampedro et al., TREES-D-08-0211 revised

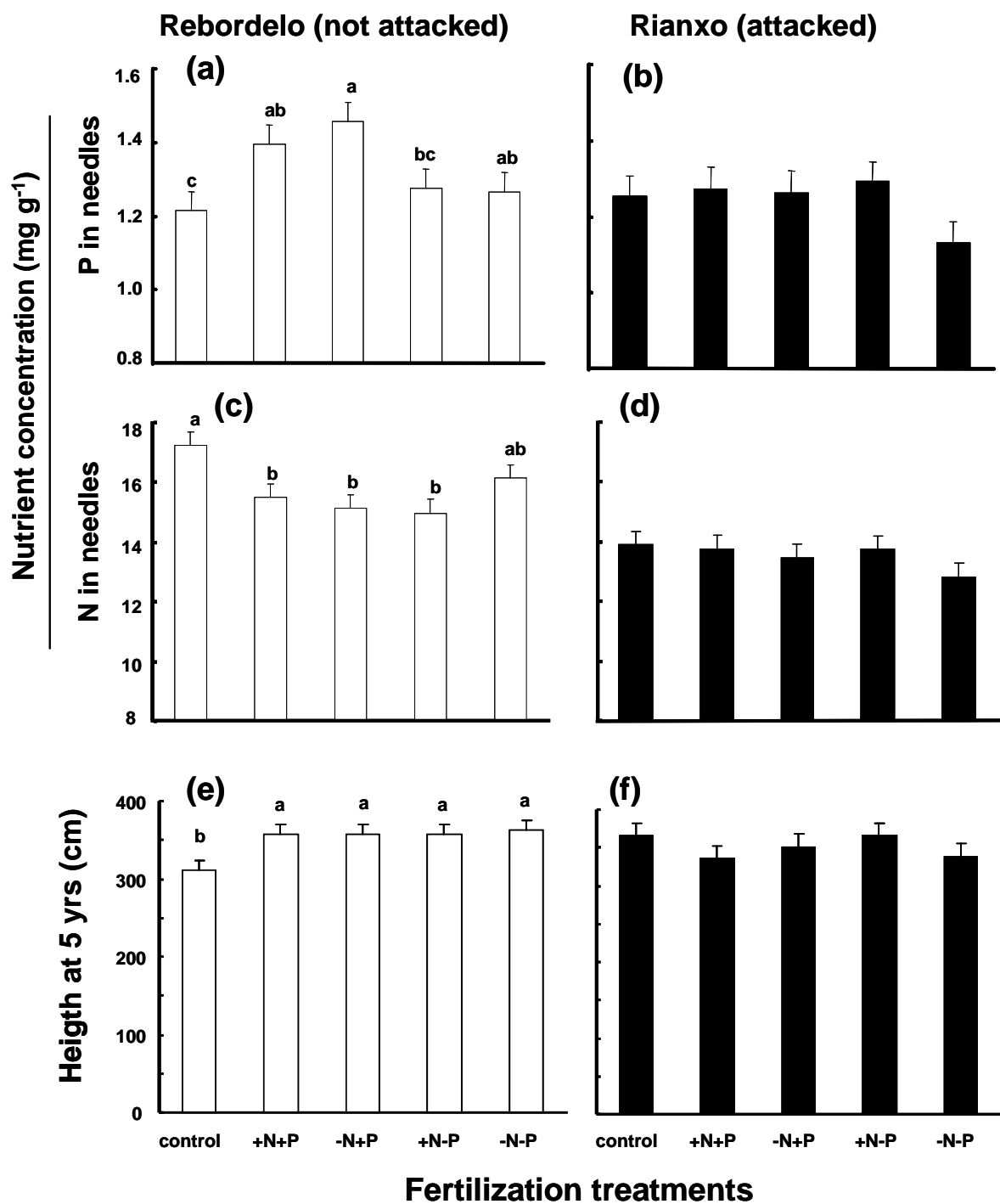


Figure 2. Sampedro et al., TREES-D-08-0211 revised

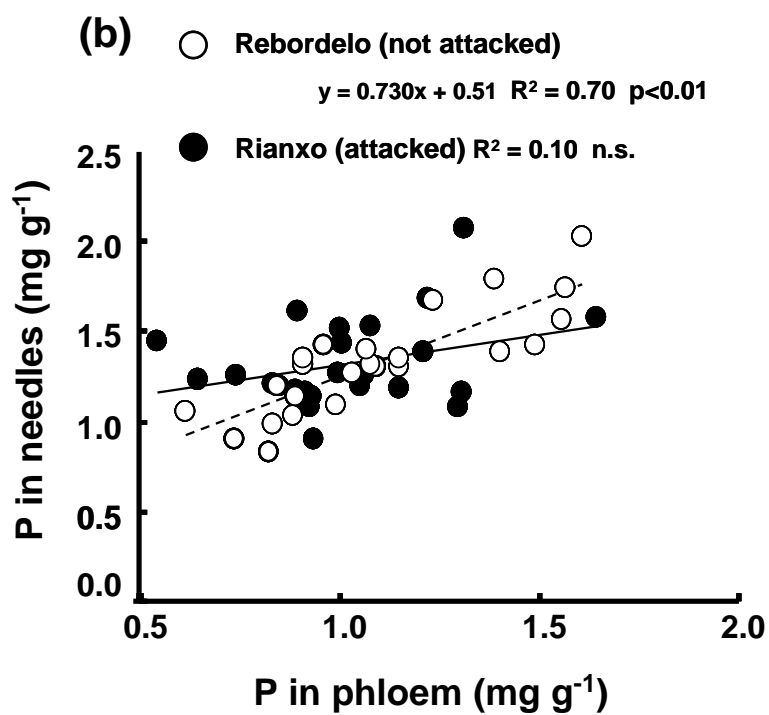
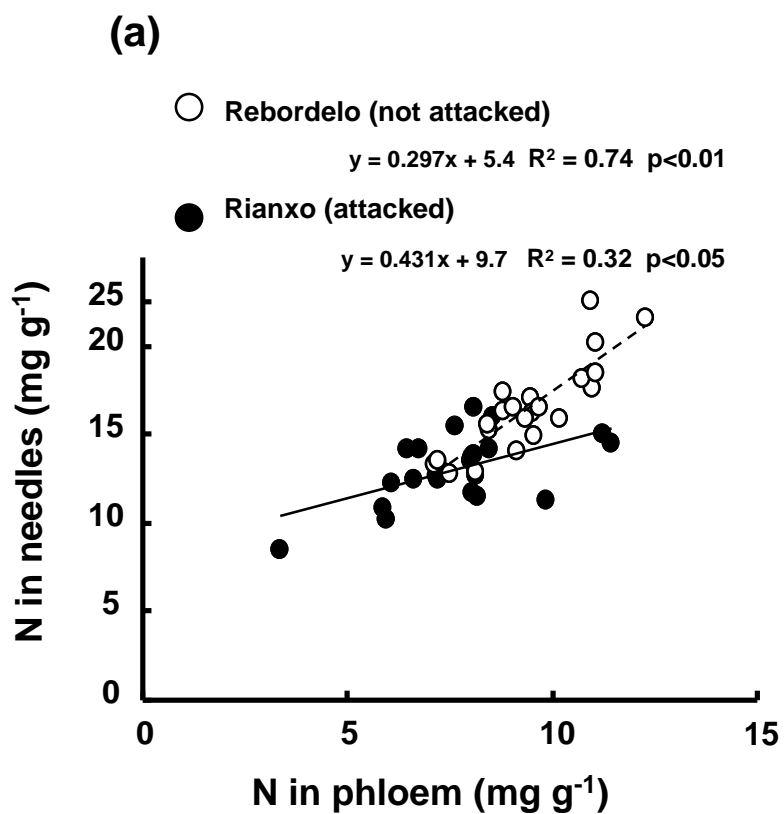


Figure 3. Sampedro et al., TREES-D-08-0211 revised