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Distribution of endemic bark beetle attacks and their physiological consequences on *Pinus halepensis*

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17 **Abstract**

18 One factor that determines a tree's resilience capacity to drought is its level of interaction with
19 aggravating biotic factors, such as bark beetles, which in turn depends on whether insect
20 populations are at an endemic or epidemic stage. In a Mediterranean natural forest ecosystem,
21 we investigated the ecophysiological characteristics of trees which (i) predispose *Pinus*
22 *halepensis* to attacks of endemic populations of bark beetles (*Tomicus destruens*) and (ii) that
23 arise as a result of these attacks. The annual life cycle of *Tomicus destruens* features an initial
24 dispersal phase of sexually mature adults towards pine trunks for the purposes of brood
25 production, and a second dispersal phase of callow adults from trunks to pine shoots for
26 sexual maturation. During a three-year survey of endemic bark beetle attacks in a stand of 178
27 trees, we found that trees targeted for trunk attacks showed a more aggregated distribution
28 pattern than those targeted for shoot attacks. Moreover, shoot-attacked trees were clearly
29 larger and taller, had lower wood density, and were less exposed to competition than non-
30 attacked trees. No differences were found between the characteristics of trunk-attacked trees
31 and non-attacked ones. We found crown defoliation, lower primary growth and alteration of
32 trunk non-structural carbon in phloem in the year following trunk attacks, although this did
33 not affect vulnerability to cavitation of the vascular system, secondary growth, wood density,
34 and xylem starch. Conversely, the health status and physiological variables of the shoot-
35 attacked trees were not altered, which may be explained by their initial vigor. We conclude
36 that the distribution of *Tomicus destruens* attacks at the plot level was independent of the
37 ecophysiological traits of the host trees. It was mainly determined by the dispersal strategy of
38 the endemic bark beetle population especially during brood production as only trunk attacks
39 significantly weakened the trees.

40 **Keywords:** bark beetle, mediterranean, endemic, *Pinus halepensis*, tree physiology, sucrose

41 **Running title:** endemic bark beetle attacks on *Pinus halepensis*

42

43 **Introduction**

44

45 Global changes are likely to have a significant impact on the distribution of many tree forest
46 species, and their associated animal and plant communities. Increases in the frequency of
47 dieback and mortality in some tree species are also expected in this context (Allen et al.,
48 2010). Such declines in tree populations are usually triggered by repeated droughts and
49 heatwaves, although individual trees can display resilience which, in some cases, enable them
50 to avoid mortality and benefit from the return of favorable conditions. One factor driving
51 resilience opportunities is the presence (or absence) of biotic aggravating factors (Manion,
52 1981; Durand-Gillmann et al., 2014). Biotic aggravating factors include numerous
53 phytophagous insect species e.g. bark beetles that commonly feed on the cambial tissues of
54 dead, recently damaged or even heavily-defended healthy trees (Paine et al., 1997). Severe
55 mountain pine beetle (*Dendroctonus ponderosae*) outbreaks can lead to up to 90% mortality
56 of the tree basal area as observed in North America (Harvey et al., 2014) while European
57 forests and plantations are regularly managed to reduce the impact of bark beetle (Klutsch et
58 al., 2009). Bark beetles are thus considered as significant disturbance agents in forested areas
59 worldwide (Raffa et al., 2008) affecting critical processes in forest dynamics and forest
60 services (Kurz et al., 2008; Hicke et al., 2012; Weed et al., 2013) with important subsequent
61 societal issues (Abrams et al., 2017; Morris et al., 2018).

62 Dispersing adult bark beetles colonize their host tree by boring an entrance hole
63 through the bark. Females dig a tunnel near the cambium in which they lay their eggs;
64 developing broods dig their own tunnel by feeding on the phloem. In conifers, tree defense
65 mechanisms against bark beetle attacks comprise a system of toxic resin ducts of variable size
66 and number, and the synthesis of defensive chemicals at the entrance site (Ferrenberg et al.,
67 2014). The resin, in addition to being toxic, presents a physical barrier that the insect needs to
68 overcome. Thus, the likelihood of a tree's survival following a bark beetle attack is closely

69 linked to the interplay between a tree's ability to mobilize its defense mechanisms and the
70 profusion of attacking bark beetles (Paine et al., 1997). Consequently, stress factors that
71 weaken a tree's defenses are usually associated with the initiation of bark beetle epidemics
72 (Marini et al., 2012). Drought is particularly likely to increase a tree's susceptibility to insect
73 attacks and to induce changes in its physiology (Gaylord et al., 2013; Netherer et al., 2015).
74 Bark beetles are inherently labile forest insect populations displaying transitions between
75 endemic states in which they reside in stands at very low densities and are expected to kill
76 only a few weakened trees, and epidemic states in which they reach very high densities over
77 large areas causing high tree mortality at both the stand and landscape scales (Kausrud et al.,
78 2012). Knowledge on the impacts of insect epidemics on tree individuals and populations has
79 benefitted from extensive research (Raffa et al., 2008; Grégoire et al., 2015), although less
80 attention has been paid to those associated with endemic states (Raffa et al., 2005; Boone et
81 al., 2011; Bleiker et al., 2014).

82 Mediterranean forests are expected to face strong pressure due to climate changes in
83 the coming decades. The frequency and intensity of droughts is likely to increase in the
84 Mediterranean (Dubrovský et al., 2014) and their co-occurrence with extreme climate-related
85 disturbances (e.g. heatwaves and storms) and induced disturbances (e.g. wildfires and pest
86 outbreaks) will be more harmful to forest health than the mean temperature elevation (IPCC,
87 2014). Additionally, insect and disease issues in Mediterranean forests can be specific rather
88 than shared with temperate ones (Lieutier et al., 2016). In drought-sensitive ecosystems, such
89 as Mediterranean forests, the study of tree and bark beetle interactions during endemic states
90 of insect populations is an appropriate effort towards effective forest management to reduce
91 the likelihood of endemic populations transitioning into epidemics, and to avoid subsequent
92 massive tree mortalities. In such a context, it is increasingly important to determine the poorly

93 known physiological characteristics that predispose a tree to attacks from Mediterranean bark
94 beetles and, to assess the consequences of endemic attacks on tree mortality.

95 In this paper, we wish to contribute toward filling these knowledge gaps by focusing
96 on the interactions between an endemic population of the pine shoot beetle *Tomicus destruens*
97 (Wollaston, 1865) and its host *Pinus halepensis* Mill. The distribution of *T. destruens* is
98 restricted to the Mediterranean basin, where it is considered the most threatening bark beetle
99 species to Mediterranean pine ecosystems (i.e. the stone pine *Pinus pinea* L., the maritime
100 pine *Pinus pinaster* Aiton, the Calabrian pine *Pinus brutia* Ten or *P. halepensis* (Faccoli et al.,
101 2008). *Tomicus destruens* is an eruptive species i.e. its populations display lengthy low-
102 density periods followed by intermittent irruptions (Lieutier et al., 2016). The *Tomicus* genus
103 is a species of particular interest as its life cycle is divided into developmental and maturation
104 phases that systematically and spatially segregate. Brood development occurs within trunks as
105 larvae feed on the phloem to reach a sexually non-mature adult stage before spreading
106 throughout the bark to disperse towards newer green shoots to mature sexually and dig a
107 feeding tunnel (Lieutier et al., 2016). These distinct and structurally different targets for bark
108 beetle feeding activity may be linked to distinct host choice strategies by the insects, and
109 distinct ecophysiological consequences for their hosts.

110 Here, we will specifically address, in a natural population of *P. halepensis*, the three
111 following objectives: (i) to characterize the spatial distribution of trunk *versus* shoot attacks
112 by an endemic population of bark beetles, (ii) to identify ecophysiological and dendrometric
113 features (tree height, needle traits, previous secondary radial growth, wood density,
114 vulnerability to cavitation, and trunk reserves) that could predispose the trees to bark beetle
115 attacks at both trunk and shoot levels, and (iii) to determine the functional consequences of
116 these attacks on crown defoliation, primary and secondary growth, phloem and xylem, and
117 Non Structural Carbon (NSC) of *P. halepensis*. We posit that the spatial distribution of *T.*

118 *destruens* attacks may differ throughout the insect's life cycle as a response to tree
119 predisposing factors that depend the insect's targets. We also posit that *T. destruens* attacks
120 may weaken defoliated and low-growth trees, and that attacks may increase defoliation,
121 decrease growth, and alter the trunk carbon and hydraulic functioning of the trees.
122

123 **Materiel and Methods**

124 **Tree-insect system**

125 The Aleppo pine, *Pinus halepensis*, is a tree species that typically grows in the dry temperate
126 areas around the Mediterranean basin from Morocco and Syria to Portugal and Greece. This
127 tree species is heliophilous, thermophilous and fire-resistant (Ducrey et al., 1996). *Pinus*
128 *halepensis* is also a polycyclic tree and the adult is known to produce up to four annual
129 growth units in the growing season, one or two (and more rarely three) from late winter to the
130 beginning of summer, and sometimes one after the summer drought (Girard et al., 2010). It is
131 also a drought-adapted species that can tolerate high xylem tension (David-Schwartz et al.,
132 2016) and implement a drought avoidance strategy by closing its stomas when water stress is
133 not yet severe (Baquedano and Castillo, 2006).

134 The pine shoot beetle, *T. destruens*, is primarily attracted by volatiles emitted from
135 fresh underbarked timber and dying or stressed pines. It takes advantage of favorable climatic
136 conditions and increased resource availability to attack healthy trees (Branco et al., 2010;
137 Lieutier et al., 2016). *T. destruens* is one of the few bark beetle species to display a continuous
138 life cycle throughout the year (Lieutier et al., 2016). This species is characterized by two
139 dispersal phases during its univoltine life cycle. Sexually immature adults emerge in spring
140 (April-May) from one to two larval cohorts (i.e. sister broods) that have developed in the
141 trunks over winter before flying towards the crowns of healthy pines and tunneling into
142 vigorous newer green shoots to complete sexual maturation. In late autumn (October-
143 November), sexually mature adults of *T. destruens* leave the shoots to seek recently cut trees
144 on which to mate and lay their eggs under the bark (Faccoli et al., 2008). The larvae feed and
145 develop in the phloem while pupation occurs in the outer bark. Adults carry spores of
146 associated fungal species (e.g. *Leptographium* spp.) that spread in the phloem and contribute
147 to wood devaluation through wood color alteration (Bezoz et al., 2015; Bois and Lieutier,

148 1997; Peverieri et al., 2006). The presence of *T. destruens* is essentially indicated by pitch
149 tubes of resin, sawdust produced by insect penetration on the trunks and green or red shoots,
150 and a medullary gallery on the ground.

151

152 **Study site**

153 This study was launched in February 2011 and conducted over a three-year period (2011-
154 2013) on a plot measuring 0.7 hectares in the communal forest of Gémenos (Bouches-du-
155 Rhône, 43° 17'16.50"N-5° 37'43.84"E, altitude 181 m, France). The forest is composed of *P.*
156 *halepensis* reaching about 15.6 m (mean DBH: 30.5 cm) and an understorey dominated by
157 *Quercus coccifera* L. This site has been locally and repeatedly infested by *T. destruens* since
158 2002. All trees in the study plot were mapped (N = 178 trees); 166 living trees and 12 dead
159 ones. Early preliminary winter inspections of trunks for *T. destruens* attacks suggested an
160 insect population in an endemic state with ca. 10% of the trees presenting pitch tubes of resin.
161 The temperature and relative humidity were recorded using HOBO Pro V2 data loggers
162 (Prosensor, France) fixed to the top of a tree in the study plot; precipitation was assessed
163 using data from Aubagne located five kilometers away. Between 2011 and 2013, the site's
164 mean annual temperature was 15.18 °C (+/- 7.26°C), the relative humidity was 66.2% (+/-
165 17.15%) and the precipitation was 660 mm (+/- 271 mm). The studied years (2011 to 2013)
166 were relatively humid corresponding to a recovery period after a long drought between 2000
167 and 2006 (Figure s1). Although this study was conducted on a sole local site, we collected
168 extensive information on the local spatial dynamics of insects, as well as the key dendrometric
169 and ecophysiological components of their host population.

170

171 **Spatial distributions of insect attacks**

172 Unlike an epidemic state during which almost all trees in a given plot are attacked by bark
173 beetles, it is relevant to assess spatial patterns of attacks in an endemic state, which implies
174 limited attacks among a majority of healthy trees. In our study plot, we aimed to identify the
175 levels of spatial aggregation of *T. destruens* attacks occurring on the trunks in the breeding
176 phase, and on the shoots in the maturation phase of the insect's life cycle. Each individual tree
177 in the plot was inspected for evidence of *T. destruens* attacks. Trees with attacked trunks were
178 identified as trees showing pitch tubes of resin on the bark's surface; these were fully counted
179 on each attacked tree. White pitch tubes corresponded to individual beetles attempting to
180 reach the phloem in the bark while orange-pink pitch tubes i.e. active attacks hereafter,
181 indicated that the beetle had reached the phloem. Trees with attacked shoots were identified
182 from shoot discoloration in the summer combined with direct shoot examination for the
183 presence of entry holes of beetles using climbing techniques. Conversely, pitch tubes of resin
184 on the trunks and attacked shoots could not be fully counted as most of them were
185 inaccessible to the observer within the tree crown. Individual tree examinations were carried
186 out in February 2011, 2012 and 2013 for trunk attacks, and between June/July 2011 and 2012
187 for shoot attacks.

188

189 **Factors predisposing *T. destruens* attacks on *P. halepensis***

190 For each tree (n = 178), we measured the tree height, tree diameter, level of competition, past
191 growth using ring width, ring wood density, tree health status, and the presence of bark beetle
192 attacks on the trunk and/or shoots. On a sub-sample of trees (n =12), we also assessed the
193 weekly diameter growth, leaf surface, Huber Value at the branch level (i.e. the ratio between
194 the leaf area and the stem diameter supporting the leaves), the Non-Structural Carbohydrate
195 (NSC) content in xylem and phloem, the leaf water potential (pre-dawn and midday), and the
196 vulnerability to embolism. According to its temporal positioning with respect to a bark beetle

197 attack, the measured physiological characteristic is either a predisposing factor to the attack or
198 a physiological consequence of this attack (Table 1). These twelve trees had similar diameters
199 (range: 25.5 - 38.2 cm); six were healthy and six had attacked trunks.

200 We calculated the level of competition experienced by a tree i (CI_i) according to its diameter
201 (dbh_i), that of its neighbor j (dbh_j), and the distance separating them (d_{ij}), considering all the
202 trees (n) located in a radius (r) around the tree i as competitors (Martin and Ek, 1984).

$$203 \quad CI_{MAEK,i} = \frac{1}{dbh_i} \sum_{j=1}^n \left(dbh_j * \exp \left[\frac{-16 * d_{ij}}{dbh_i + dbh_j} \right] \right)$$

204 Past radial growth and wood density were assessed using micro-densitometric and ring-width
205 profiles. One core per tree was taken in winter 2012-2013 using an increment borer at breast
206 height (DBH; 1.30 m); this was performed perpendicular to the slope to avoid sampling bias.

207 To estimate the trees' ring widths, cores were taken using a razor blade and then digitalized
208 with a high-resolution scanner. Ring width (RW; millimeters per year) chronologies were
209 measured at a precision of 0.01 mm and were cross-dated using CDendro v5.3 and
210 CooRecorder 5.3 software (Cybis Elektronik & Data AB, Sweden). For each tree, the ring-
211 width chronologies were obtained and cross-dated according to a reference chronology in
212 order to delimit missing or false rings, and thus standardize the radial growth data by
213 removing the age effect. To estimate the wood density, two-millimeter segments were taken
214 from the cores using twin saws, and the resin from each core was removed with pentane. The
215 samples were X-rayed and the resulting radiographic films were digitized with a high-
216 resolution scanner after resin removal. Density levels were associated with the gray levels in
217 the image by means of a previously established calibration curve. Windendro® software
218 (Regent Instruments Inc., Canada) was used to delimit the annual growth ring and calculate
219 the micro-densitometric profiles. Only 115 trees were retained in the end as some cores were
220 damaged during sawing, and some images were blurred during analysis. The sub-sample of

221 cored trees shows a similar distribution of defoliation levels to the whole sample (Figure s2).
222 A detrend analysis was performed using a spline function (DplR package in R) to remove age
223 effects. Common statistics were estimated on raw ring width series (rw: ring with, rwi: early
224 wood width, rwf: late wood width, wd: wood density, wdi: wood density of early wood, wdf:
225 wood density of late wood) and detrended ring widths (autoCorr: ring width autocorrelation
226 and Gini).

227 To characterize the trees' health status, we followed a protocol established by the
228 French Department of Forest Health (Ministry of Agriculture, Agribusiness and Forest,
229 France, 2013). This consists of evaluating tree crown damage based on the percentage of
230 needle chlorosis and defoliation. For two summer periods (2011 and 2012), all the trees were
231 observed with binoculars from the ground by the same two operators. Four groups of trees
232 were created based on their percentages of needle chlorosis and defoliation:

- 233 - Group 0: healthy trees (less than 20% crown defoliation and needle chlorosis)
- 234 - Group 1: trees in moderate decline (between 20 and 40% crown defoliation and needle
235 chlorosis)
- 236 - Group 2: trees in serious decline (more than 40% crown defoliation and needle chlorosis)
- 237 - Group 4: dead trees (completely defoliated).

238 On the subset of twelve trees, measurements of the surface area, length and weight of
239 the needles, and the diameters of the shoots allowed the calculation of the Leaf Mass Area
240 (LMA) and Huber Value (HV). A growth retrospective analysis (Barthélémy and Caraglio,
241 2007) was performed on shoots taken by a climber at four positions in the crown (top, upper,
242 middle and lower) and the four cardinal points (north, south, east and west). The data was
243 recorded using Multi Tree Scale formalism (Godin and Caraglio, 1998) with AMAPstudio
244 (Griffon and de Coligny, 2014). The length and number of annual shoot growth units in 2011
245 and 2012 were measured in June 2012.

246

247 **Physiological consequences of *T. destruens* attacks on *P. halepensis***

248 We measured the starch concentration and soluble sugars (fructose, glucose, sucrose
249 and total sugars) in the sub-sample of the twelve previously described trees in order to assess
250 potential interferences of *T. destruens* attacks with circulation of sap and nutrients in their
251 host.

252 Trees were sampled twice in June (28th, doy 178) and in October (5th, doy 278) 2011.
253 For each tree, a trunk xylem core (5 mm diameter) and a small disk of bark (12 mm diameter)
254 were extracted at a height of 1.3 m. Samples were transported to the laboratory in a chiller
255 where they were immediately placed in a freezer (-80 °C). Following lyophilization, samples
256 were finely ground using a ball mill (MM 301, Retsch, Germany). Soluble sugars and starch
257 were extracted from 10 mg of the dry matter obtained from the phloem and the outermost 1.5
258 cm of the xylem. Soluble sugars were extracted twice with 1 ml of boiling 80% ethanol.
259 These 2 ml were then combined and centrifuged. The supernatants and pellets containing
260 starch were dried in a centrifugal vacuum concentrator to remove the ethanol (Centrivap,
261 Labconco, USA). The soluble sugars and pellets containing starch were then rehydrated with
262 0.02 mol l⁻¹ NaOH. Glucose, fructose and sucrose were assessed enzymatically using the
263 method described by Barbaroux et al., (2003). The concentrations were then added to
264 calculate the concentration of total soluble sugars. Starch was quantified as the glucose
265 equivalent using the enzymatic method described for soluble sugars. The concentrations of
266 total starch, and total soluble sugars (i.e. fructose, glucose and saccharose) were expressed in
267 grams of reserve compound per 100 g of dry matter (g 100 g DW⁻¹).

268 Finally, leaf water potential and xylem vulnerability to embolism were assessed. Pre-
269 dawn leaf water potential was measured in 2011, 2012 and 2013 in summer before sunrise.
270 Two shoots per tree, with a stem of 2 cm minimum, were collected. The samples were placed

271 in individual bags in the dark and then put into a chiller to maintain their inner branch
272 pressure (avoiding all contact with ice). Leaf water potential was measured with a Scholander
273 bomb or a pressure chamber (Scholander et al., 1965). In the laboratory (and within one hour
274 of collection), vulnerability to embolism was determined through vulnerability curves
275 obtained in 2013 and 2014 on trees surveyed for leaf water potential, leaf traits and weekly
276 growth, and on 24 shoot-attacked and healthy trees respectively. Vulnerability curves and
277 xylem-specific hydraulic conductivity were assessed using an air-injection method in
278 Avignon for the first sample in 2013, and with the CAVITRON at INRA Bordeaux for the
279 second in 2014 (see Delzon et al., 2010 for methodological details). In previous studies, P50
280 was found to be the same using both techniques (Ennajeh et al., 2011). Stems were wrapped
281 in moist paper and placed in plastic bags to avoid water loss through transpiration. They were
282 immediately sent to the CAVIPLACE laboratory where they were stored at 4 °C prior to
283 measurements (two weeks maximum in this case). For the measurements, the shoots were
284 debarked and cut under water to a standard length of 27 cm; both ends were pared with a
285 razor blade. Samples were not flushed with water in order to avoid possible effects of air-
286 seeding fatigue due to stretching or degrading the pit membranes during the previous
287 embolism events. A solution of ultra-pure deionized water containing 10mM KCl and 1mM
288 CaCl₂ was used as the reference ionic solution. Hydraulic conductivities at every rotation
289 speed were measured using Cavisoft software (v. 5.2, University of Bordeaux) in order to
290 obtain vulnerability curves corresponding to the percentage loss of hydraulic conductivity as a
291 function of xylem pressure.

292

293 **Statistical analysis**

294 Spatial patterns of trunk and shoot attacks by *T. destruens* were inferred from a multi-
295 distance spatial cluster analysis with Ripley's K-function (Ripley, 1976), which has been

296 widely applied to distribution patterns of herbs, forest trees and sedentary animals (Fortin et
297 al., 2016; Haase, 1995; Pringle et al., 2010). We used Ripley's method for the univariate point
298 pattern analysis in a two-dimensional space based on the distances between tree individuals to
299 test whether a process (i.e. trunk or shoot attacks) was clustered at one or multiple different
300 distances within the pine plot. Observed repartitions of attacked trees were compared to
301 simulated repartitions obtained by randomly distributing the same number of attacks among
302 trees in the stand. Spatial patterns of insect attacks were inferred by defining both upper and
303 lower 95% confidence thresholds for the observed statistic $K(t)$ of each process of attack at
304 the inter-individual distance t based on 1,000 simulations of the process. Spatial distributions
305 of insect attacks are considered as being: clustered when $K(t)$ is above the upper confidence
306 limit, having complete spatial randomness when $K(t)$ is within the 95% confidence threshold,
307 and regular when $K(t)$ falls below the lower confidence threshold (Diggle, 2013; Fortin et al.,
308 2016). These analyses were performed with the spatstat package in R (Baddeley et al., 2011).

309 To assess the predisposing factors, an initial analysis was carried out factor by factor
310 using a logistic regression to separate the trees into three classes (non-attacked, shoot-attacked
311 and trunk-attacked trees). Multinomial logistic regressions were performed using the
312 'multinom' function (nnet package in R; Ripley and Venables, 2016). To show the factors that
313 discriminate the three classes, we calculated the coefficient of the logistic regression with
314 non-attacked trees being taken as the reference class. We tested whether the regressed
315 coefficients differ from zero using Wald z-tests from AER package in R (Kleiber and Zeileis,
316 2019).

$$317 \quad \textit{Class}_{tree} = \textit{Factor}_{tree} + \epsilon_{tree}$$

318 Tests on the sub-samples of the initial population were carried out using rank statistics
319 (Wilcoxon rank sum test) to compensate for the small population size.

$$Class_{tree} = \sum_k^n Factor k_{tree} + \epsilon_{tree}$$

321

322 Concerning the effects of attacks on physiological or growth traits, a simple analysis of
 323 variance was carried out with the diameter as a co-variable when it had a significant effect.

$$324 \quad Traits_{tree} = Class_{tree} + DBH_{tree} + \epsilon_{tree}$$

325

326 **Results**

327 **Spatial distributions of insect attacks**

328 Individual living tree examinations for *T. destruens* attacks indicated a low to almost
 329 null prevalence of trunk attacks in 2011 (N =19, 11.5% of total living trees), 2012 (N = 3,
 330 1.8%) and 2013 (N =1, 0.6%). In 2011, the mean number (+SE) of white and orange-pink
 331 resin pitch tubes per tree was 88 (+20) and 32 (+10, 32% of total pitch tubes) respectively.
 332 Pitch tubes were not counted in 2012 and 2013 due to insufficient numbers of attacked trees.
 333 The prevalence of shoot attacks in 2011 and in 2012 was higher than prevalence of trunk
 334 attacks (N = 21 i.e. 11.8% and N = 62 i.e. 34.8% respectively). Two trees attacked on their
 335 trunks in 2011 were found to be dead in 2012. The multi-distance spatial cluster analysis,
 336 using Ripley's K-function, showed significant clustering of pine trunk attacks by *T. destruens*
 337 at distance radii of 5-10 m and 20-25 m in 2011 (Fig. 2a). In contrast, distributions of shoot
 338 attacks in the spring of 2011 and 2012 showed no deviation from complete spatial
 339 randomness thus suggesting the absence of an aggregative process of shoot attacks during the
 340 sexual maturation phase on healthy trees (Fig. 2b-c).

341

342 **Factors predisposing *T. destruens* attacks on *P. halepensis***

343 The plot density was low (208 stem.ha⁻¹) with a Relative Density Index of 0.5 when
 344 using the equations of Charru et al., (2012). Consequently, competition explained only 6% of

345 the growth variation between trees suggesting this competition was probably driven more by
346 light than water as the correlation between growth and competition no longer increased
347 beyond a seven-meter radius (Figure s3).

348 To estimate the dendrometric predisposing factors for bark beetle attacks, we gathered
349 the two-year attack data by separating trunk-attacked and shoot-attacked trees (Figure 3ab).
350 Shoot attacks occurred on trees with greater diameters ($p = 0.0019$), heights ($p = 0.0668$) and
351 less competition ($p = 0.0589$) than non-attacked trees. Trunk attacks occurred on trees
352 statistically similar to non-attacked trees, except for a stating significance ($p = 0.082$) effect
353 for higher tree height. Attacks on trunks and shoots occurred on trees whose growth was
354 greater than that of non-attacked trees from 2005 (Figure 4a). Attacks on shoots were more
355 common on trees with an aptitude for taking advantage of good years especially in spring for
356 early wood ($p = 0.014$ for RWI and $p = 0.149$ for RWF) and whose wood density was lower
357 (Figure 4b), whereas trunk attacks occurred on trees that had mostly poorer growth in
358 unfavorable years (Figure 4a).

359

360 **Physiological consequences of *T. destruens* attacks on *P. halepensis***

361 Larger diameter trees showed less defoliation and less abnormal coloration in 2011
362 and 2012. Therefore, diameter was almost always taken as a co-variable for analyzing the
363 effects of attacks on defoliation and discoloration. Trees attacked on their trunks in 2011
364 showed higher defoliation in 2011 and in 2012 (Table 2), which suggests that the effects of
365 the attack persisted over time and even increased. Trunk attacks had the same negative effects
366 in terms of needle discoloration yet. Surprisingly, trees attacked on their shoots (in 2011 and
367 2012) were not found to be significantly more defoliated or discolored than average (Table 2)
368 following the attack.

369 Shoot growth on trunk-attacked trees in 2011 was significantly reduced i.e. by 21%
370 after the attack ($p = 7.81e-05$; figure 5). However, this growth decline began in 2010, which
371 suggests that the poor growth of these shoots might also be an indicator of weaknesses in the
372 shoot and therefore a marker of a predisposing factor to insect attacks (Figure 6). The trunk
373 attack also had a significant effect on the annual shoot growth in 2012 ($p = 0.007$) and on the
374 number of growth units of the 2012 annual shoot ($p = 0.0461$). In contrast, the 2011 trunk
375 attacks had no effect on the surfaces of the needles ($p = 0.472$), their weight ($p = 0.652$) or the
376 leaf per mass area ($p = 0.823$).

377
378 Trunk attacks in 2011 had no effect on secondary growth measured through circumferences at
379 1.30 m ($p=0.41$) or ring widths in 2011 ($p=0.91$) and 2012 ($p=0.84$). However, the bark beetle
380 attack may not have had an effect yet, because there may be a delay between the attack and
381 the effect on growth. Only a slight negative effect on the initial density of 2012 wood was
382 found ($p = 0.0526$). The 2011 shoot attacks had no effect except for a negative effect on 2011
383 summer wood ring width ($p = 0.09$). The lack of effect of trunk attacks on secondary growth
384 was confirmed by dendromicrometer analyzes (Figure 6), except a retraction of trunks found
385 slightly stronger during drought for trunk-attacked trees.

386
387 Tree diameter is not linked to water potential and vulnerability to embolism.
388 Therefore, it has not been taken into account as a co-variable in the following analyzes. The
389 shoots sampled for vulnerability curve measurements were sufficiently long (> 15 cm) to
390 represent more than five years of growth. Vulnerability to embolism, measured in 2013 and
391 2014 for 2011 trunk-attacked trees and in 2012 for shoot-attacked trees, may be considered
392 both as a predisposing factor for an attack and as a consequence of an attack. The P50 values
393 ranged from -7.04 MPa to -2.88 Mpa with an average value of -4.85 Mpa. Neither the trees

394 attacked on the shoots in 2012 ($p = 0.6475$) nor the trees attacked on the trunks in 2011 ($p =$
395 0.8798) showed significant differences in the xylem vulnerability to embolism (P50, pressure
396 inducing 50% loss of conductivity loss) compared to non-attacked trees (Figure 7a). Although
397 the P50 values were similar, the trunk-attacked trees appeared to be slightly more vulnerable
398 to embolism at the onset of water stress (Figure s4). Conductivity (K_{smax}) was not affected
399 by trunk attacks ($p = 0.16$) or by shoot attacks ($p = 0.47$). In addition, neither the pre-dawn
400 water potential ($p = 0.872$) nor the midday water potential ($p = 0.9$) of trunk-attacked trees
401 differed from those of unaffected trees (Figure 7b). Some of the trees whose water potential
402 was surveyed from 2011, were also attacked on their shoots in 2012. There was no effect of
403 attacks on shoots in 2012 nor on the midday potential ($p = 0.757$) or on the pre-dawn potential
404 ($p = 0.881$).

405 In contrast, in June 2011 trunk-attacked trees had higher starch concentrations in the
406 phloem and tended to have lower fructose and glucose phloem concentrations than non-
407 attacked trees (Figure 8). While non-attacked trees showed a typical decrease between June
408 and October of the different types of sugars (except for sucrose), trunk-attacked trees
409 displayed an opposite pattern for glucose and fructose. Contrary to non-attacked trees, glucose
410 and fructose xylem concentrations showed a clear decrease in attacked trees. However,
411 regardless of the sugar and date, the concentrations were very low in the xylem.

412

413

414 **Discussion**

415

416 **The determinants of the spatial distribution of *T. destruens* attacks**

417 In all, we counted 14 dead trees i.e. 7.8% of the surveyed trees over the three study
418 years. The observation of the overall low prevalence of both trunk and shoot attacks by *T.*
419 *destruens* confirmed that the bark beetle population was in an endemic state in the study plot.
420 This low prevalence can be explained by the fact that we studied these attacks during a
421 succession of favorable growth years with relatively moderate droughts. Trunk attacks
422 associated with the insects' breeding period showed an aggregative spatial pattern, while
423 shoot attacks showed almost complete spatial randomness in the insects' sexual maturation
424 phase. Moreover, trunk attacks occurred on trees statistically similar to non-attacked ones
425 (based on measured functional traits) while shoot attacks principally occurred on trees with a
426 greater diameter and height, low wood density and less competition.

427 These results suggest that the distribution of *T. destruens* attacks in an endemic state
428 were not a consequence of the distribution of the health status of the trees but rather the
429 dispersal strategies of the bark beetles, which change during different phases in their life
430 cycle. For bark beetles, one of the major limitations of fitness at low population density is the
431 inability to successfully attack trunks of healthy trees exhibiting fully expressed resistance
432 mechanisms. This implies a constraint on insects' foraging for the location of more poorly
433 protected resources for breeding (Kausrud et al., 2012). The predominant strategy among
434 most bark beetle groups is based on pheromone-mediated cooperative behavior to accumulate
435 on a tree in order to overcome its defenses (Pitman, 1969; Raffa et al., 2015), although *T.*
436 *destruens* is one exception (Lieutier et al., 2015). Trunk colonization by pine shoot beetles
437 proceeds from repeated tree interceptions by pioneer beetles through random flights and by
438 the subsequent increase in tree attractivity for new colonizers due to the host volatiles (e.g. α -
439 pinene, terpinolene, ethanol) stimulated by the pioneer attacks (Byers, 1992; Lieutier et al.,

440 2015). This is likely to generate aggregative patterns of individual trees subjected to trunk
441 attacks if the attracted pioneer congeners fail to locate the target tree and land on surrounding
442 ones. Our results for *T. destruens* were consistent with aggregative attacks of other pine
443 species by the closely related species *T. piniperda* during its breeding phase (Lieutier et al.,
444 2015; Sauvard et al., 1987).

445 During their sexual maturation phase, bark beetles attacked shoots of trees with larger
446 diameters and with healthier conditions. This suggests that target trees were those with more
447 needles that potentially provide the insects with more abundant high-quality maturation sites.
448 Vigorous shoots release an alluring blend of α -pinene and β -myrcene (Faccoli et al., 2008),
449 and *T. destruens* shows a significant preference for and increased performance on well-
450 watered pine shoots than on water-stressed ones (Branco et al., 2010). However, whether *T.*
451 *destruens* actually seeks out compounds in vigorous shoots that would be expressly involved
452 in sexual maturation is still unknown. Paradoxically, the bark beetle strategy with fewer
453 attacks on defoliated and weakened trees, could homogenize the health status of the entire tree
454 population. Moreover, larger diameter trees also have a larger crown area (Forrester et al.,
455 2017) and are therefore more likely to be attacked during the sexual maturation phase of
456 insects at low population density. This higher shoot accessibility for the insects is a more
457 likely driver of their dispersal strategy than shoot resistance where a lower resin production
458 than in the trunks may be less constraining for insects (Krokene, 2015).

459 460 ***T. destruens* attacks on trunks affect tree water and carbon functioning**

461 The mean P50 values found in this study are very similar to the ones reported in
462 different *P. halepensis* provenances growing in arid environments (David-Schwartz et al.,
463 2016). The vascular system was unaffected by trunk attacks as suggested by the lack of
464 differences in xylem vulnerability to embolism and water potential. Nevertheless, we found

465 that trunk-attacked trees showed greater trunk shrinkage during drought episodes. This
466 suggests that attacks can affect tree capacitance and thus their resistance to very pronounced
467 droughts (Salomón et al., 2017). While trunk attacks did not affect secondary growth they did
468 lead to a decrease in primary growth, as well as higher defoliation and foliage discoloration in
469 the year following the attack; this generally reduced photosynthesis (Galiano et al., 2011).

470 When significant amounts of stored resin are exuded from the resin ducts in the
471 phloem and xylem, the intruding beetles may be repelled or 'pitched out'. This resin is highly
472 toxic to bark-beetle eggs and larvae and inhibits fungal growth. In lodgepole pines, a series of
473 biochemical alterations are associated with insect attacks including a pronounced increase in
474 monoterpene levels, the conversion of a monoterpene composition into more toxic and
475 repellent compounds, and the accumulation of heartwood phenolics (Raffa and Berryman,
476 1982). Prior to and during the expression of active wound physiology, the metabolically
477 passive flow of resin from severed ducts can delay beetle progression into the cambium thus
478 giving the trees more time to respond to attacks by employing other chemical defense
479 mechanisms (Raffa and Berryman, 1982). The oleoresin synthesized by pines is derived from
480 isoprenoid units (isopentenyl diphosphate); these terpenes are mostly biosynthesized in the
481 cambium zone through the classic mevalonic acid pathway from acetyl-CoA in cytoplasm, or
482 through the MEP(2-C-Methyl-D-erythritol 4-phosphate) pathway in chloroplast. However,
483 exudation of the constitutive resin depends on fructose and glucose availability within the
484 duct system, and the viscosity of the oleoresin (Christiansen et al., 1987). This carbon-heavy
485 defense mechanism can have implications for the tree's carbon balance (Martínez-Vilalta,
486 2014). In this study, it might explain the decrease in fructose and glucose concentrations on
487 attacked trees in the spring following the winter trunk attacks. Such lower levels of fructose
488 and glucose could stimulate their production by local hydrolysis of the starch. This may be of
489 benefit to insects as a feeding substrate (Raffa et al., 2015), support induced resinosis

490 following insect attacks (Krokene, 2015), and participate in maintaining ring growth even if
491 the phloem transport is locally disrupted. As the lateral movement of sugars between the
492 xylem and the phloem have already been reported (Aubry et al., 2019), changes in fructose
493 and glucose levels in the phloem can also induce changes in the xylem. Moreover, in the
494 Pinaceae, the traumatic ducts are formed in the xylem (Franceschi et al., 2005), which may
495 also explain the sucrose changes in xylem. Finally, as gymnosperm have high lipid content
496 (Kozlowski and Pallardy, 1996), further quantifications of lipid reserve dynamics would be of
497 critical interest in terms of examining their potential role as an insect food resource and in
498 terms of growth sustainment.

499

500 **Conclusions and perspectives**

501 In this study, we show that a population of *T. destruens* at an endemic stage may not
502 necessarily attack weakened host trees, and that notably they specifically targeted the shoots
503 of the most vigorous trees during the sexual maturation phase of their life cycle. We also
504 suggest that the aggregative nature of trunk attacks during the breeding phase may be due to
505 insect foraging behavior in a context of rare suitable hosts, rather than depicting clusters of
506 trees weakened by unfavorable micro-scale conditions (e.g. micro-edaphic). At such a low
507 insect population level, attacks appeared to have little or no effect on tree health and we found
508 a low tree mortality rate. In an endemic context, such weak effects of insect attacks support
509 the strong resilience potential of trees which may have enough starch to face the physiological
510 consequences of trunk attacks and that may not be affected by the subsequent falling of
511 attacked shoots. This study was carried out on one focus pine population and our results
512 would benefit from larger scale investigations, as well as being paralleled with a similar
513 approach in the context of an insect epidemic. However, we are able to provide relevant
514 support for the few studies showing that the processes at play during bark beetle endemic

515 phases are in clear contrast to those occurring during epidemic phases (Raffa et al., 2005;
516 Boone et al., 2011; Bleiker et al., 2014) and which generally result in overwhelmed tree-
517 resistance mechanisms and resilience abilities (Paine et al., 1997; Kausrud et al., 2012). This
518 work supports the potential conflicts between tree resilience abilities to endemic bark beetle
519 attacks, and the current most highly-recommended management of bark beetle risk in forests,
520 which involves the removal of any attacked trees to prevent the occurrence of bark beetle
521 outbreaks (Fettig and Hilszczański, 2015).

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742 **Table 1:** List of measurements done at stand scale or on a sub-sample to assess predisposing
 743 factors explaining the attacks of *Tomicus destruens* and their subsequent effects on *Pinus*
 744 *halepensis* health in 2011 and 2012 in southeastern France.

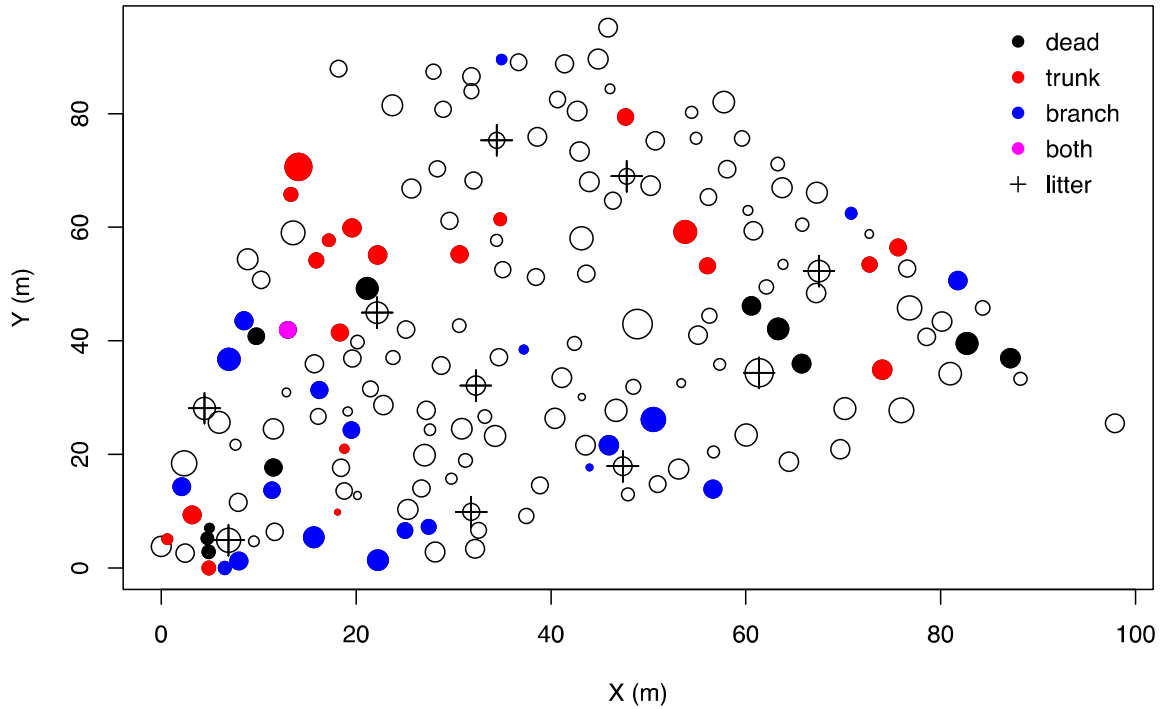
	Stand		Sub-sample	
	Trunk	Shoot	Trunk	Shoot
	2011-2012	2011-2012	2011-2012	2011-2012
Predisposing factors	DBH ₂₀₁₁	DBH ₂₀₁₁	Ψ_{50}	Ψ_{50}
	Height ₂₀₁₁	Height ₂₀₁₁		
	Competition	Competition		
	Leaf Color ₂₀₁₁	Leaf Color ₂₀₁₁		
	Defoliation ₂₀₁₁	Defoliation ₂₀₁₁		
	RW ₁₉₅₀₋₂₀₁₀	RW ₁₉₅₀₋₂₀₁₀		
	WD ₉₅₀₋₂₀₁₀	WD ₁₉₅₀₋₂₀₁₀		
Effect variables	Leaf Color ₂₀₁₂	Leaf Color ₂₀₁₂	Ψ_{50}	Ψ_{50}
	Defoliation ₂₀₁₂	Defoliation ₂₀₁₂	Weekly growth	Weekly
	RW ₂₀₁₁₋₂₀₁₂	RW ₂₀₁₁₋₂₀₁₂	Sugars	growth
	$\rho_{\text{wood}2011-2012}$	$\rho_{\text{wood}2011-2012}$		

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746 **Table 2:** Physiological effects (percentage and p value using a linear model) of *Tomicus*
 747 *destruens* attacks on trunk and shoots on Defoliation (DEF) or Abnormal Color (AC) (black)
 748 observed on *Pinus halepensis* individuals in 2011 and 2012 southeastern France. In grey, the
 749 factors that significantly not predispose to the shoot trunk attack. † indicates that the tree
 750 diameter has a significant effect and is taken as a co-variable in the linear model

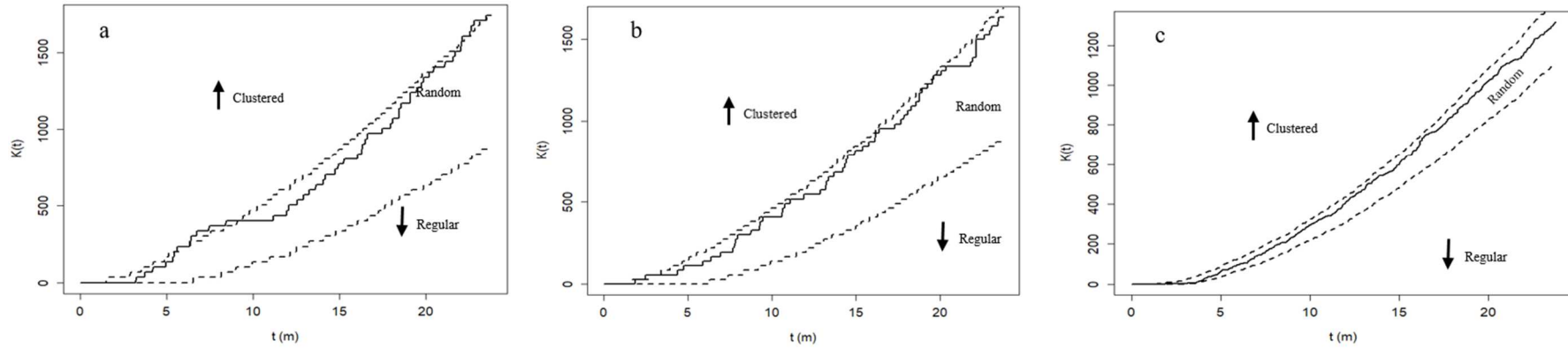
	DEF 2011	DEF 2012	AC 2011	AC 2012
Average (%)	21.53	24.76	2.63	6.53
trunk attack 2011	24.74 (7.93e-2) [†]	32.63 (1.51e-2) [†]	5.53 (7.6e-2) [†]	16.05 (8.5e-3) [†]
trunk attack 2012	ns	40 (2.35e-3) [†]	ns	26.25 (3.41e-4) [†]
shoot attack 2011	ns (0.75) [†]	ns (0.87) [†]	ns (0.30) [†]	ns (0.23) [†]
shoot attack 2012	** (2.1e-2)	21.13 (9.7e-3)	ns	ns (0.39)

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 754 **Figure 1:** Map of *Pinus halepensis* individuals in the Gemenos study site, Southern France,
 755 including trees that died before 2011 (black filled circle), trunk-attacked trees by *Tomicus*
 756 *destruens* in 2011 or 2012 (red filled circle), shoot-attacked trees in 2011 or 2012 (blue filled
 757 circle), and one tree attacked on both trunk and shoots (magenta filled circle). Circle size is
 758 proportional to the tree diameter at breast height. Litter traps implemented on the site are
 759 figured as black crosses.

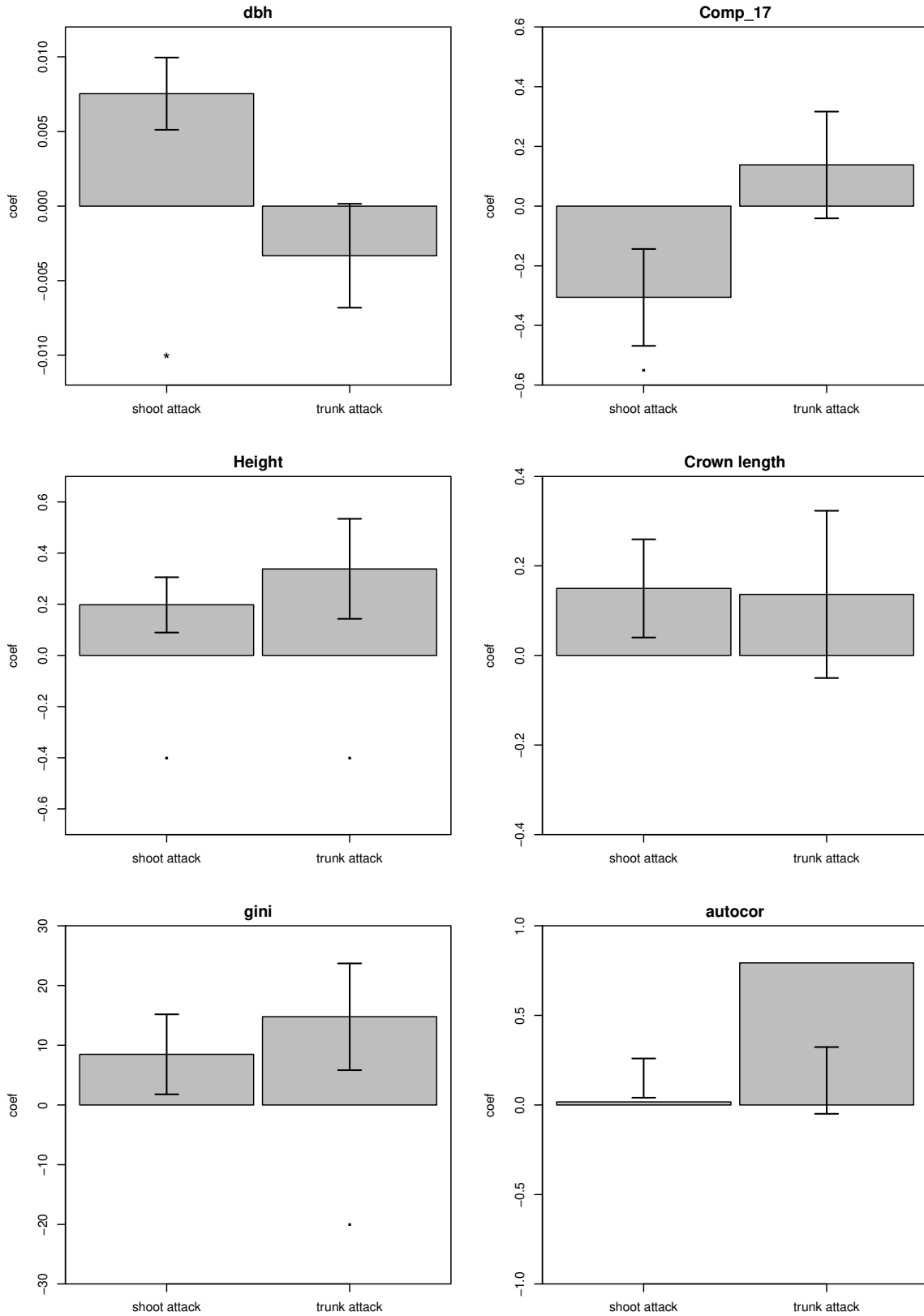
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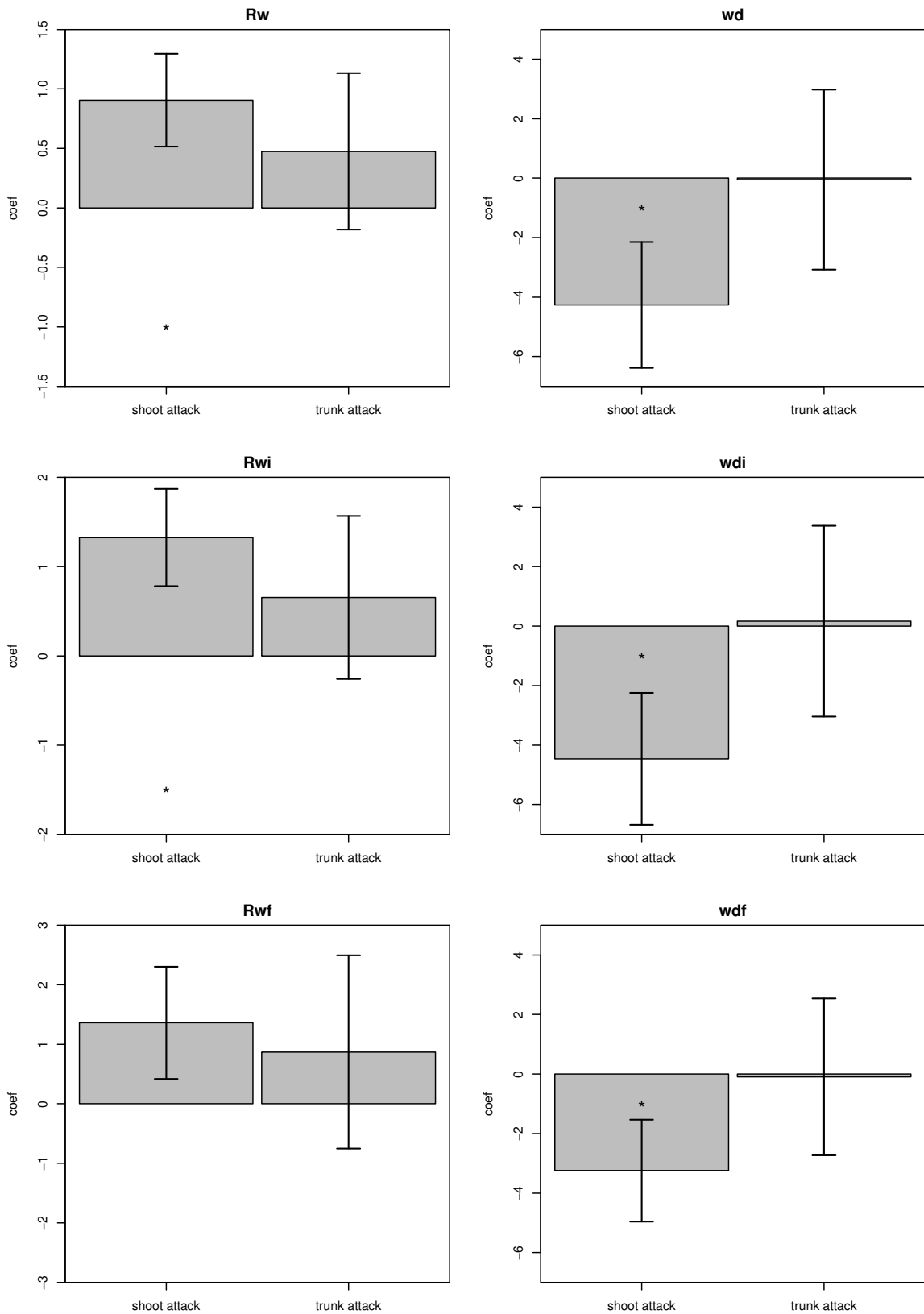
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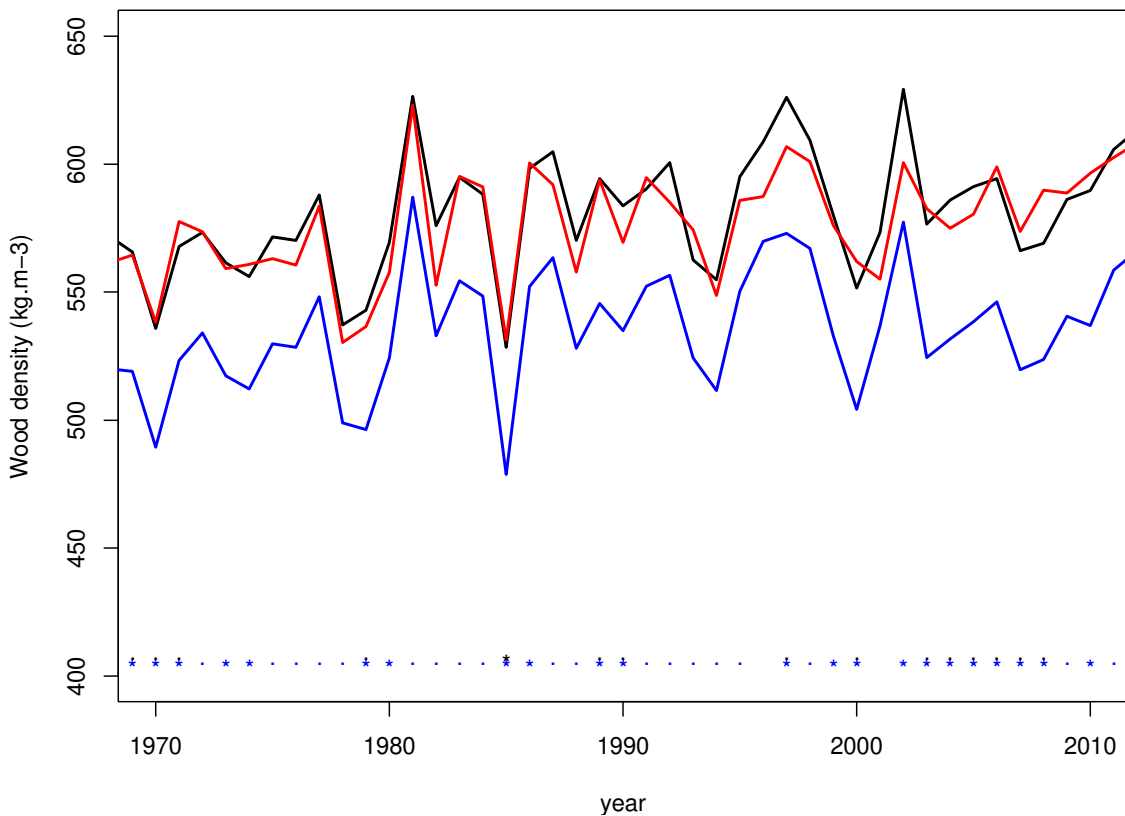
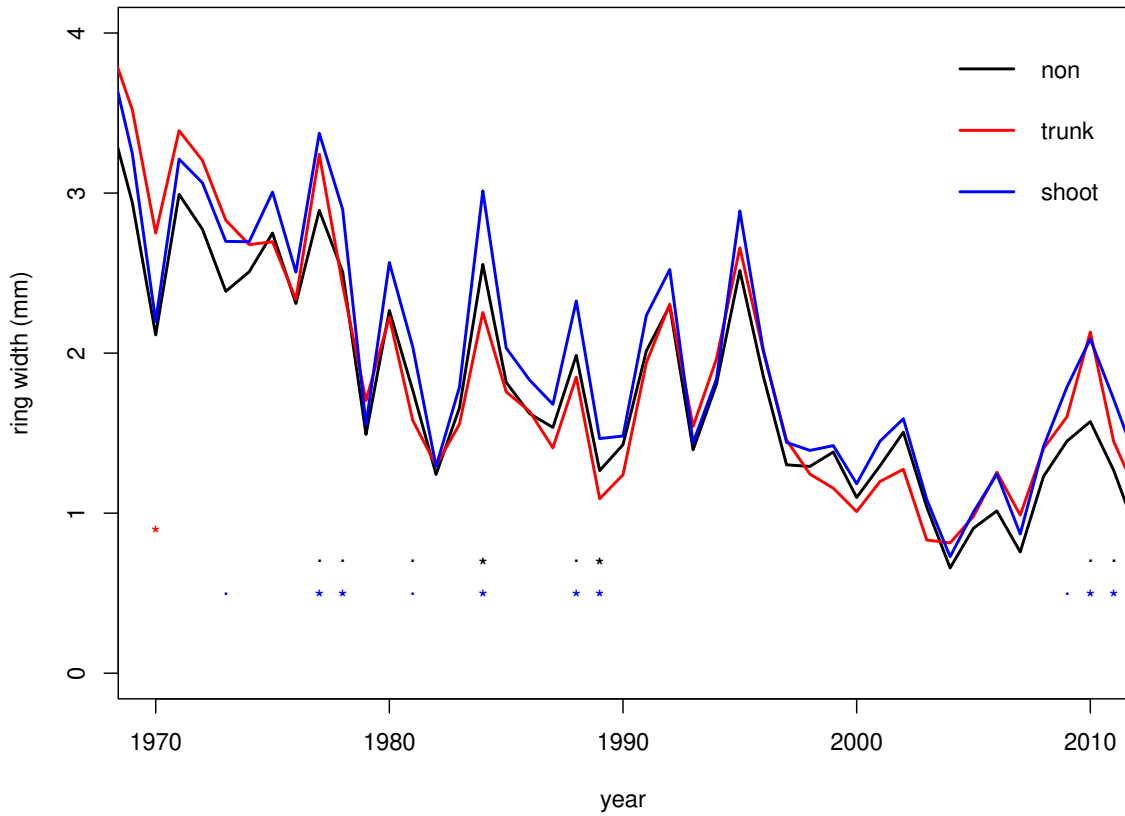
763 **Figure 2.** Univariate spatial pattern analysis using the Ripley's K-function for distributions of *Tomicus destruens* attacks in a *Pinus halepensis*
764 population in Southern France. The figure shows the observed statistic $K(t)$ for trunk attacks for the insect's breeding phase in fall 2010 (a, N=19
765 attacked trees), and shoot attacks for the insect's sexual maturation phases in springs 2011 (b, N=21) and 2012 (c, N=62), plotted against distance
766 between trees t . Black lines give the values of $K(t)$ and dotted lines give their 95% confidence envelopes. Spatial distributions of insect attacks
767 are considered: clustered when $K(t)$ is in the area above the upper confidence limit, random when $K(t)$ is inside the 95% confidence envelope,
768 regular when $K(t)$ is below the lower confidence limit.



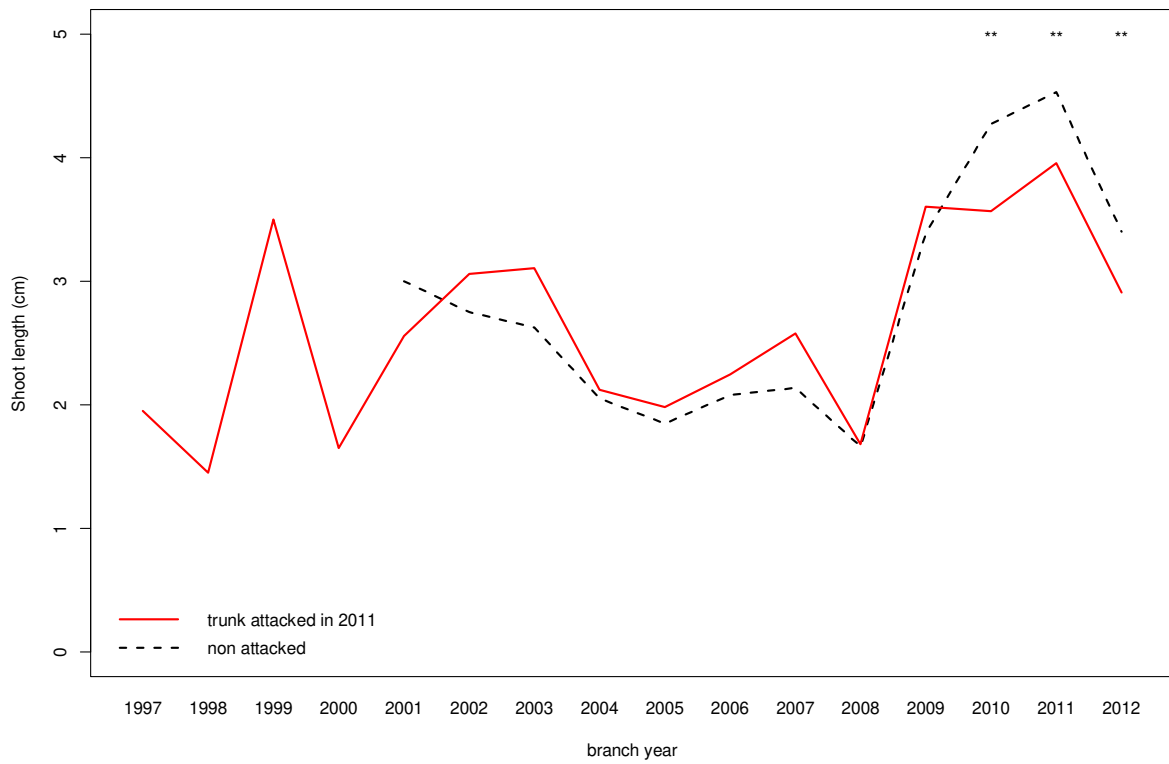
770 **Figure 3a:** Dendrometric factors predisposing *Tomicus destruens* attacks in a *Pinus*
 771 *halepensis* population in Southern France. Coefficient of the logistic regression analysis, non-
 772 attacked trees being taken as a reference. A star indicates that the coefficient is significantly
 773 different from the reference with a p-value < 0.05 and a point with a p-value < 0.1.
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 776 **Figure 3b:** Dendrometric factors predisposing *Tomicus destruens* attacks in a *Pinus*
 777 *halepensis* population in Southern France. Coefficient of the logistic regression analysis, non-
 778 attacked trees being taken as a reference. A star indicates that the coefficient is significantly
 779 different from the reference with a p-value < 0.05 and a point with a p-value < 0.1.

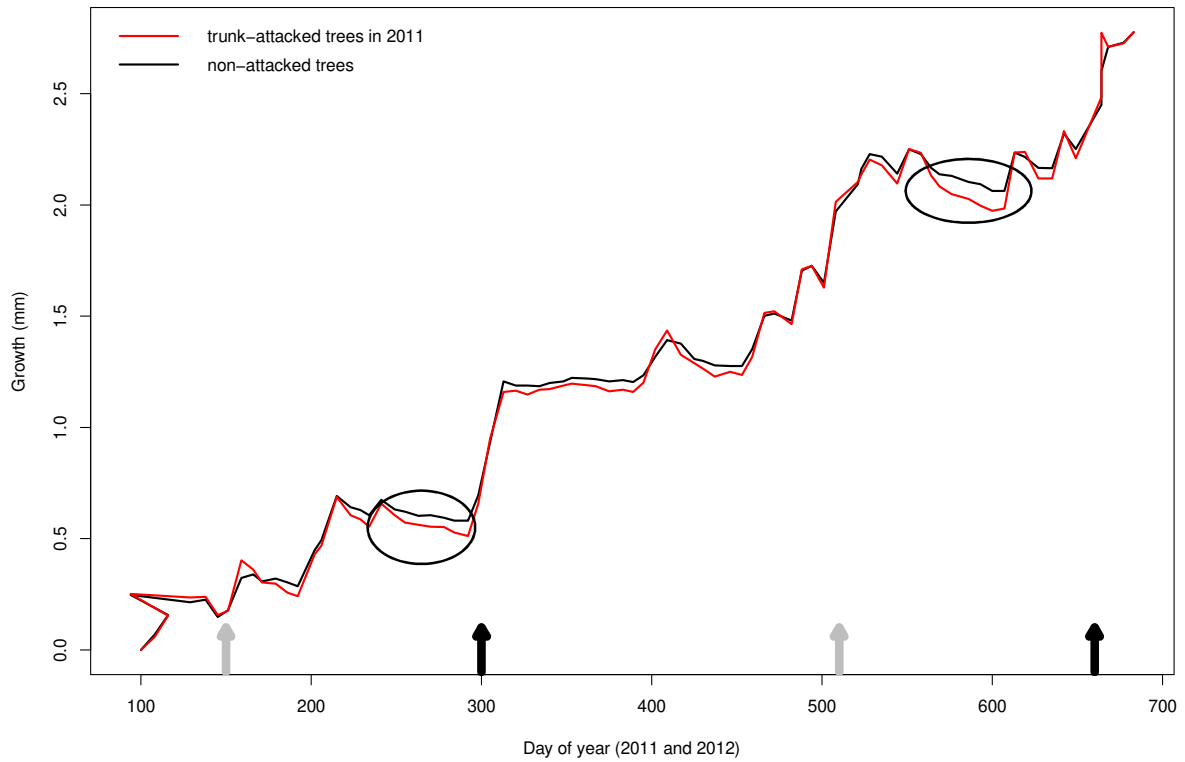


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 781 **Figure 4:** Past growth (ring width in mm) and wood density of non-attacked (black), shoot
 782 attacked (red) and trunk attacked (blue) individuals of *Pinus halepensis* in southeastern
 783 France. Significant differences between trunk attacked trees and non-attacked trees in red (*
 784 at 5% and at 10%). Significant differences between trunk attacked trees and non-attacked
 785 trees in blue. Whole significant differences in black



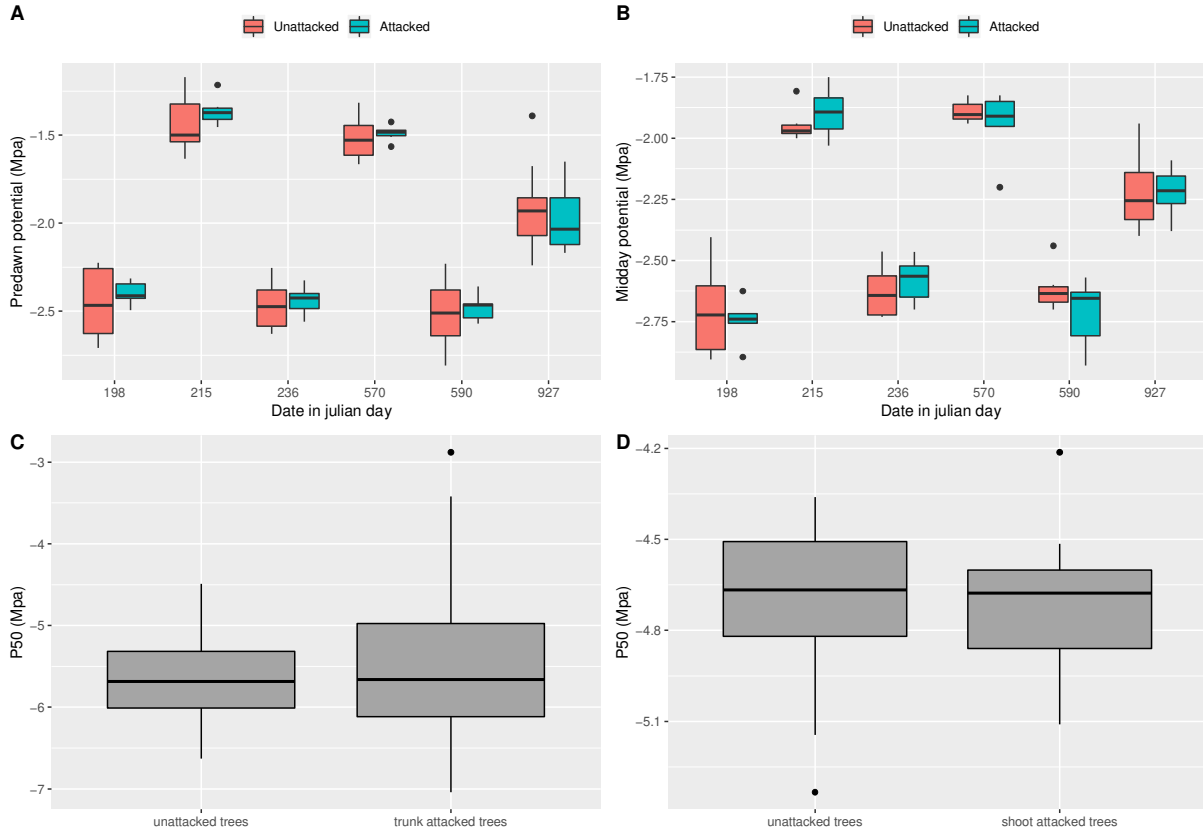
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Figure 5: Shoot length (cm) of *Pinus halepensis* individuals that were attacked and non-attacked by *Tomiscus destruens* in 2011 in southeastern France.



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Figure 6: Effect of *Tomicus destruens* trunk attacks on *Pinus halepensis* weekly growth measured using hand-held micro-dendrometers in 2011 in southeastern France. The black ellipses represent the period of shrinkage of the trunk. The black arrow represents the trunks attacks and the grey arrows the shoot attacks.



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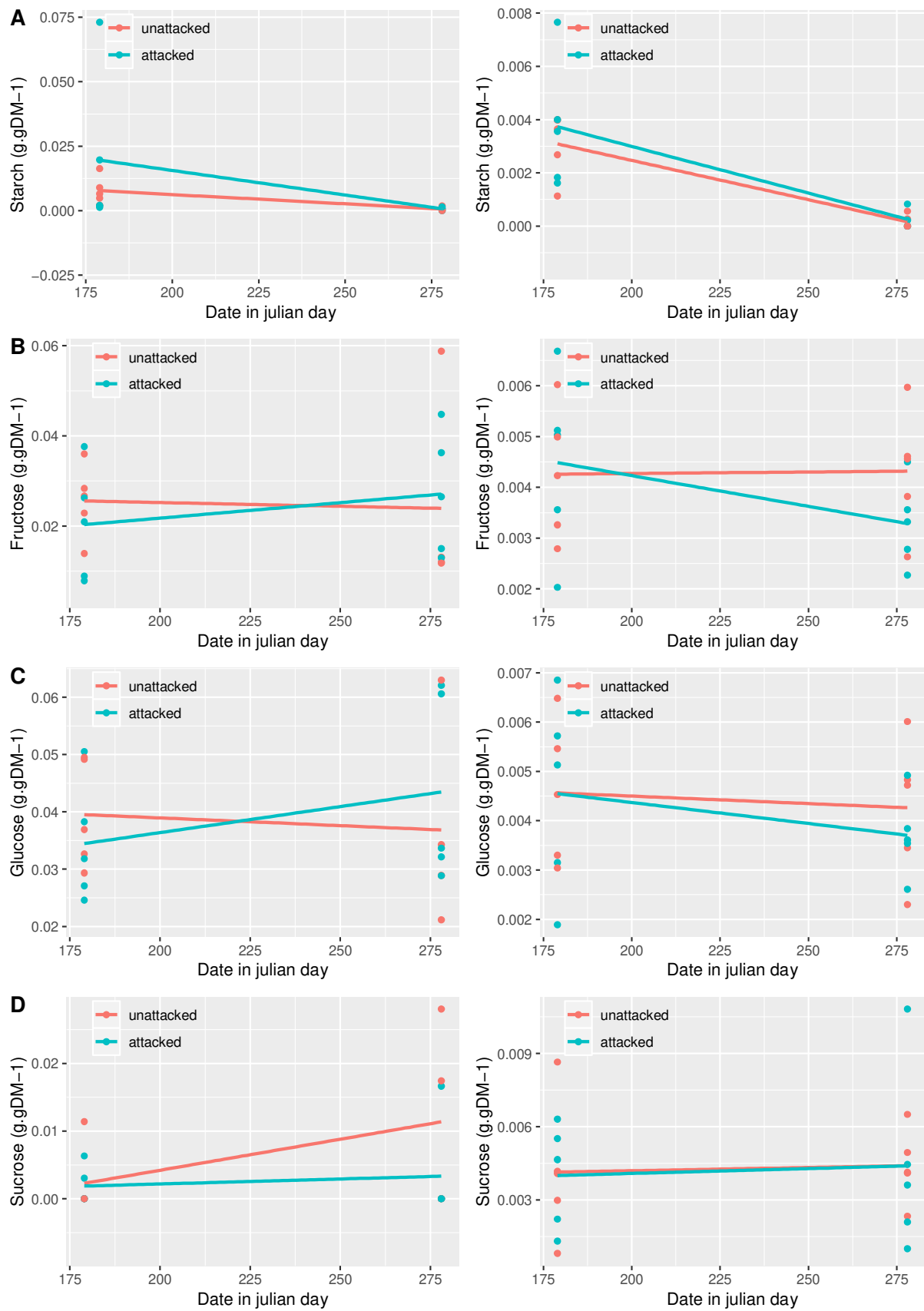
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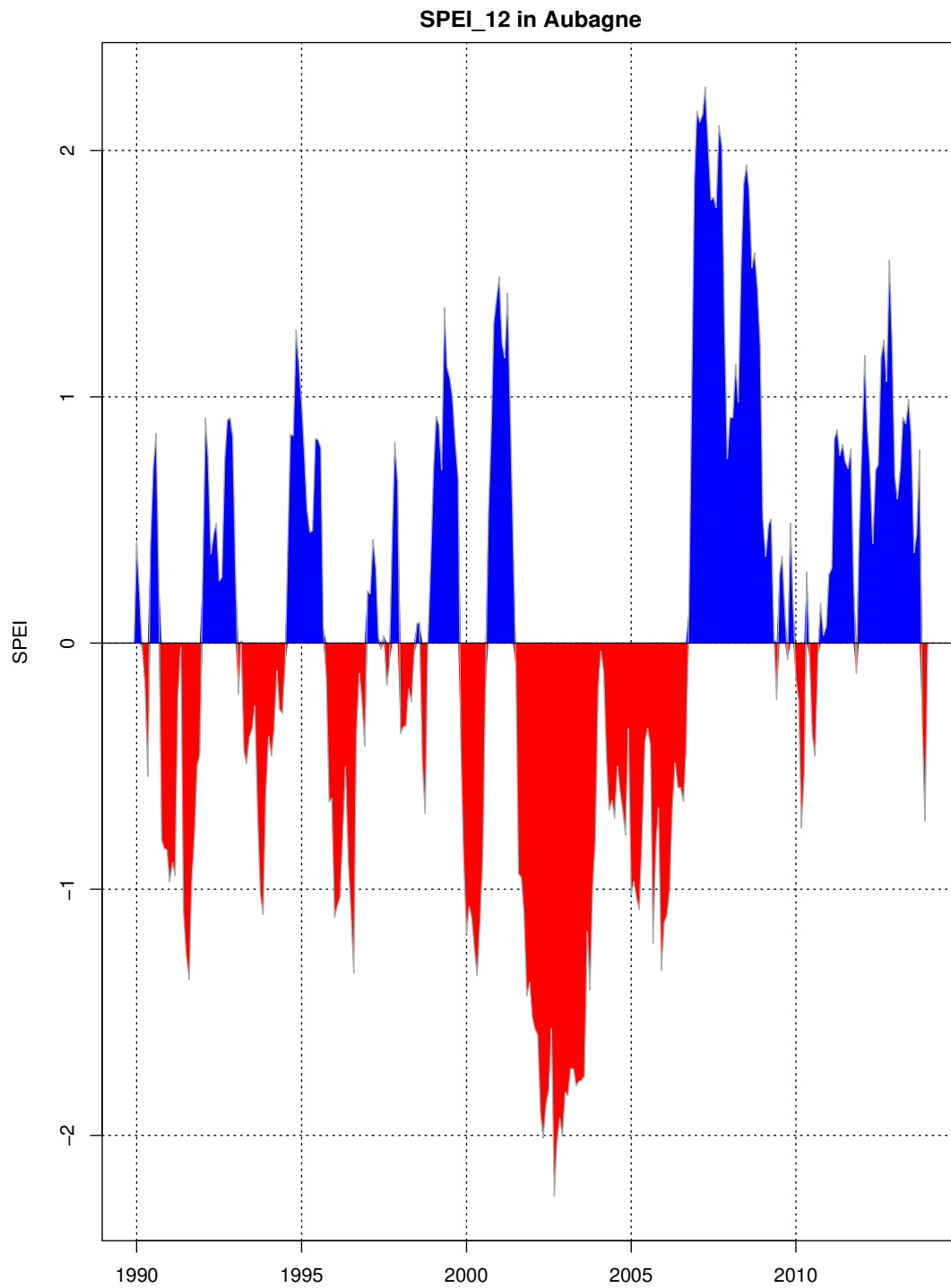
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Figure 7: (a, b) Effect of 2011 trunk attacks on predawn and midday water potentials dynamics (Mpa) on *Pinus Halepensis* trees. (c, d) Effect of trunk attacks (in 2011) and shoot attacks (in 2012) by *Tomicus destruens* on *Pinus Halepensis* xylem water potential inducing 50% of conductivity (P50 in Mpa).



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Figure 8: Non structural carbohydrates concentration (g g_{DM}⁻¹, starch, fructose, glucose, sucrose) in *Pinus halepensis* trunk ploem (left) and xylem (right) before and after trunk attacks in 2011 for trees attacked by *Tomicus destruens* (red long dotted line) and non-attacked (black dotted line).

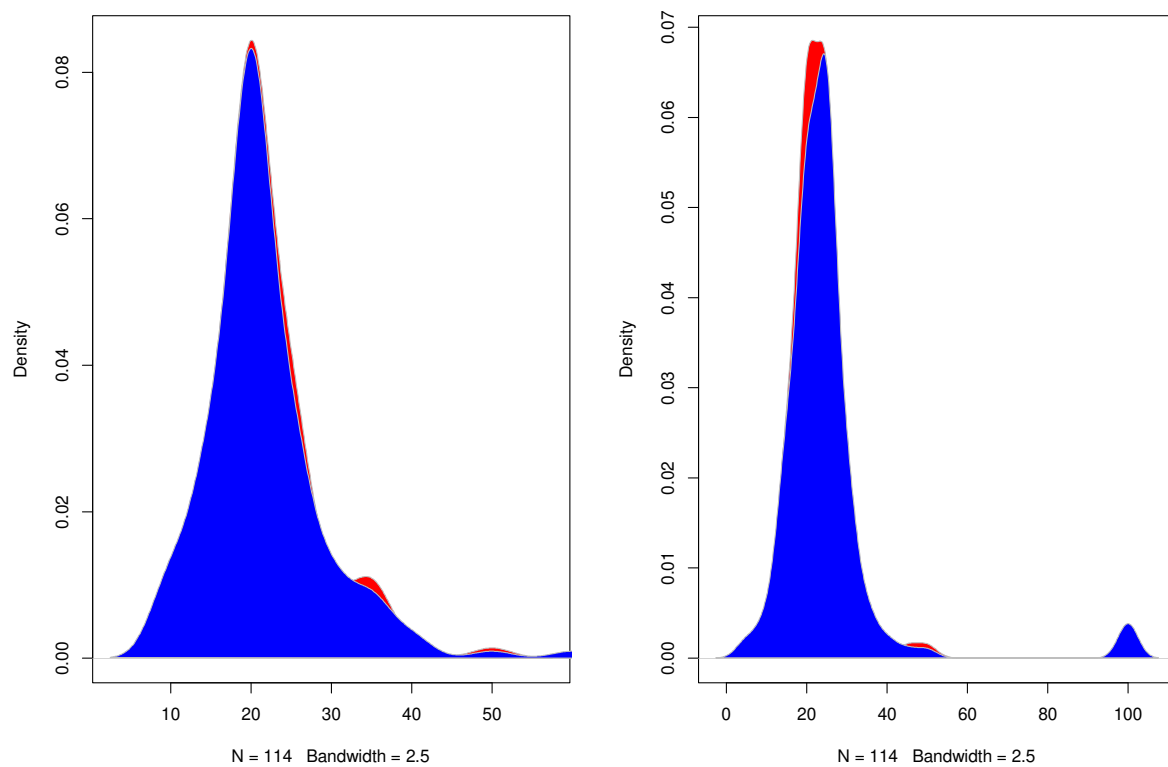


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811 **Figure s1:** Dynamics of 12-months Standardized Precipitation-Evapotranspiration Index
812 measured in Aubagne, a locality located 5 km from the study site. In red the drought periods
813 and in blue the wet periods.

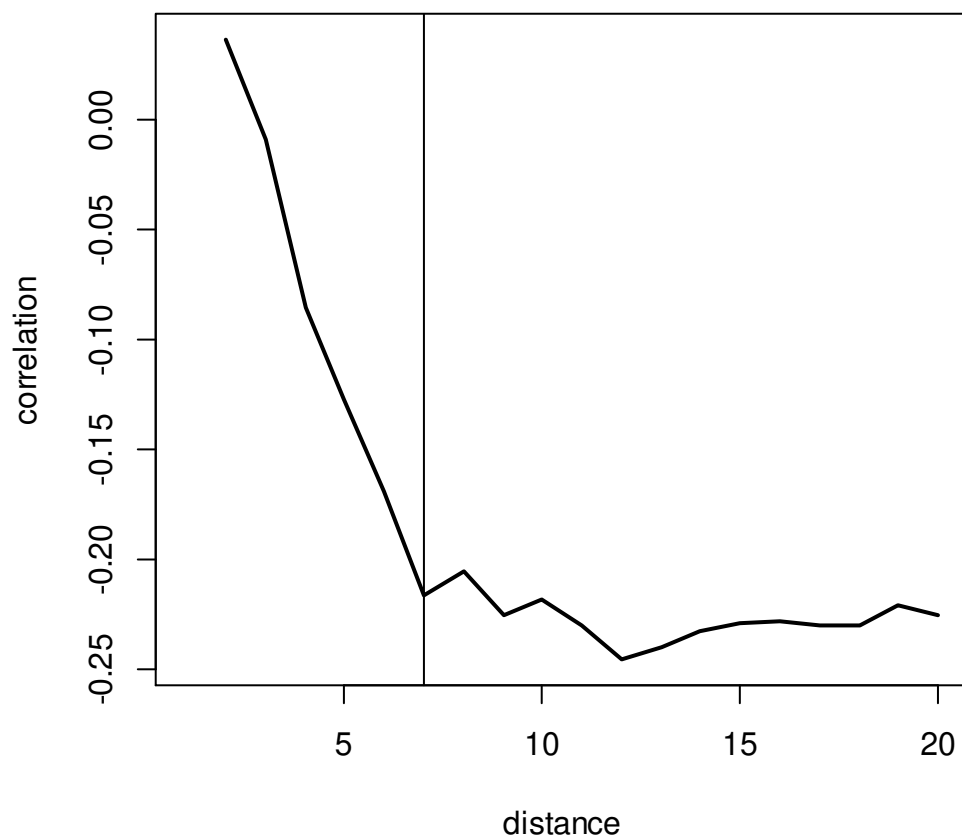
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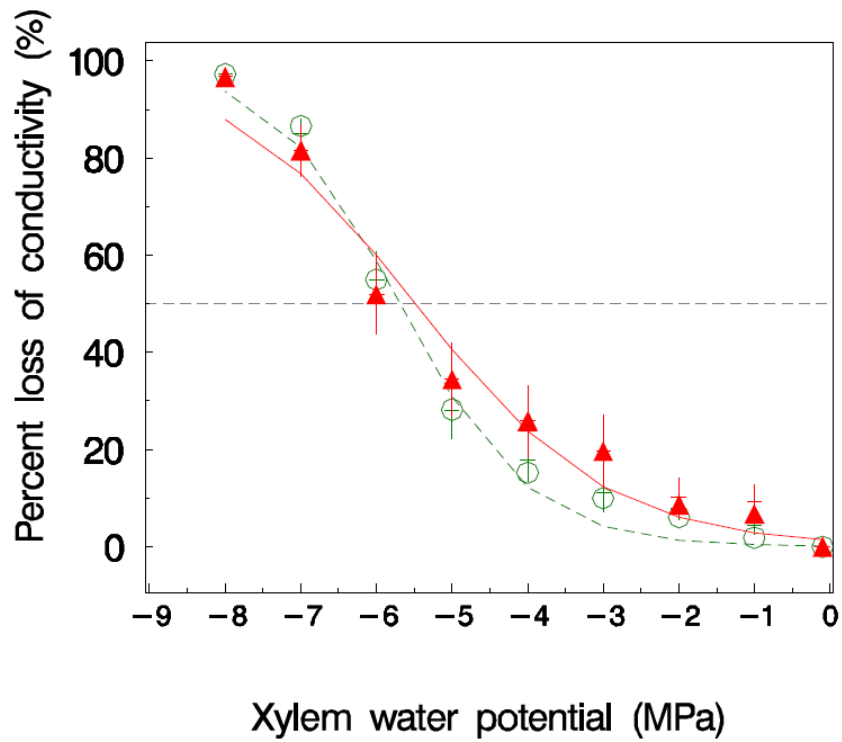


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Figure s2: Comparison of defoliation class distributions between the subsample of the all monitored *Pinus halepensis* trees (blue) and the subsample of *Pinus halepensis* trees, whose cores were analyzed (red).



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824 **Figure S3:** correlation coefficients (r) between the Martin-Ek competition index at a given
825 distance of competitive trees and growth increment in a southeastern French *pinus halepensis*
826 population between 2011 and 2012.
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Figures s4: Percent loss of hydraulic conductivity based on xylem pressure in *Pinus halepensis* in southeastern France in 2011. Full red triangles and empty green circles represent trees attacked by *Tomicus destruens* on the trunk and non-attacked trees, respectively.