



**HAL**  
open science

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

Hendrik Davi, Marion Durand-Gillmann, Claire Damesin, Sylvain Delzon, Cathleen Petit, Philippe P. Rozenberg, Sylvie-Annabel Sabatier, Joël Chadoeuf, Bernard Boutte, Thomas Boivin

► **To cite this version:**

Hendrik Davi, Marion Durand-Gillmann, Claire Damesin, Sylvain Delzon, Cathleen Petit, et al.. Distribution of endemic bark beetle attacks and their physiological consequences on *Pinus halepensis*. Forest Ecology and Management, 2020, 469, pp.118187. 10.1016/j.foreco.2020.118187 . hal-02877316

**HAL Id: hal-02877316**

**<https://hal.inrae.fr/hal-02877316>**

Submitted on 6 Jan 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



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<sup>-1</sup> 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<sup>-1</sup>).

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 CaCl<sub>2</sub> 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).

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

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.

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

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

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

## Results

### Spatial distributions of insect attacks

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

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

The plot density was low (208 stem.ha<sup>-1</sup>) with a Relative Density Index of 0.5 when 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.  $\alpha$ -  
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  $\alpha$ -pinene and  $\beta$ -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).



522        **Acknowledgements**

523        We would like to thank Alice Delaporte for the sugar measurements, Bruno Lavigne for  
524        the hydraulic measurements, and the technicians, William Brunetto, Nicolas Mariotte and  
525        Florence Courdier, for the sanitary and dendrometric measurements. This project was  
526        funded by the Department of Forest Health of the French Ministry of Agriculture, the  
527        CNRS EC2CO CITRYX project and the LABEX OTMED (n° ANR-11LABX-0061).

528

- 530 Abrams, J., Huber-Stearns, H., Bone, C., Grummon, C., Moseley, C., 2017. Adaptation to a  
531 landscape-scale mountain pine beetle epidemic in the era of networked governance: the  
532 enduring importance of bureaucratic institutions. *Ecol. Soc.* 22. [https://doi.org/10.5751/ES-](https://doi.org/10.5751/ES-09717-220422)  
533 09717-220422
- 534 Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M.,  
535 Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H. (Ted), Gonzalez, P., Fensham, R.,  
536 Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running, S.W., Semerci, A.,  
537 Cobb, N., 2010. A global overview of drought and heat-induced tree mortality reveals  
538 emerging climate change risks for forests. *For. Ecol. Manag.* 259, 660–684.  
539 <https://doi.org/10.1016/j.foreco.2009.09.001>
- 540 Aubry, E., Dinant, S., Vilaine, F., Bellini, C., Le Hir, R., 2019. Lateral Transport of Organic  
541 and Inorganic Solutes. *Plants Basel Switz.* 8. <https://doi.org/10.3390/plants8010020>
- 542 Baddeley, A., Turner, R., Beale, C., Bernhardt, R., Biggerstaff, B., Bivand, R., Bonneu, F.,  
543 Burgos, J., Byers, S., Chang, Y.M., 2011. Package ‘spatstat.’
- 544 Baquedano, F.J., Castillo, F.J., 2006. Comparative ecophysiological effects of drought on  
545 seedlings of the Mediterranean water-saver <Emphasis Type="Italic">Pinus  
546 halepensis</Emphasis> and water-spenders <Emphasis Type="Italic">Quercus  
547 coccifera</Emphasis> and <Emphasis Type="Italic">Quercus ilex</Emphasis>. *Trees* 20,  
548 689. <https://doi.org/10.1007/s00468-006-0084-0>
- 549 Barbaroux, C., Bréda, N., Dufrêne, E., 2003. Distribution of above-ground and below-ground  
550 carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petraea*  
551 and *Fagus sylvatica*). *New Phytol.* 157, 605–615. [https://doi.org/10.1046/j.1469-](https://doi.org/10.1046/j.1469-8137.2003.00681.x)  
552 8137.2003.00681.x
- 553 Barthélémy, D., Caraglio, Y., 2007. Plant Architecture: A Dynamic, Multilevel and  
554 Comprehensive Approach to Plant Form, Structure and Ontogeny. *Ann. Bot.* 99, 375–407.  
555 <https://doi.org/10.1093/aob/mcl260>
- 556 Bezos, D., Martínez-Álvarez, P., Diez, J.J., Fernández, M.M., 2015. The pine shoot beetle  
557 *Tomicus piniperda* as a plausible vector of *Fusarium circinatum* in northern Spain. *Ann. For.*  
558 *Sci.* 72, 1079–1088. <https://doi.org/10.1007/s13595-015-0515-4>
- 559 Bleiker, K.P., O’Brien, M.R., Smith, G.D., Carroll, A.L., 2014. Characterisation of attacks  
560 made by the mountain pine beetle (Coleoptera: Curculionidae) during its endemic population  
561 phase. *Can. Entomol.* 146, 271–284. <https://doi.org/10.4039/tce.2013.71>
- 562 Bois, E. (Institut N. de la R.A., Lieutier, F., 1997. Phenolic response of Scots pine clones to  
563 inoculation with *Leptographium wingfieldii*, a fungus associated with *Tomicus piniperda*.  
564 *Plant Physiol. Biochem. Fr.*
- 565 Boone, C.K., Aukema, B.H., Bohlmann, J., Carroll, A.L., Raffa, K.F., 2011. Efficacy of tree  
566 defense physiology varies with bark beetle population density: a basis for positive feedback in  
567 eruptive species. *Can. J. For. Res.* 41, 1174–1188. <https://doi.org/10.1139/x11-041>
- 568 Branco, M., Pereira, J.S., Mateus, E., Tavares, C., Paiva, M.R., 2010. Water stress affects  
569 *Tomicus destruens* host pine preference and performance during the shoot feeding phase.  
570 *Ann. For. Sci.* 67, 608–608. <https://doi.org/10.1051/forest/201021>
- 571 Byers, J.A., 1992. Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliatus*,  
572 and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes. *J.*  
573 *Chem. Ecol.* 18, 2385–2402. <https://doi.org/10.1007/BF00984957>
- 574 Charru, M., Seynave, I., Morneau, F., Rivoire, M., Bontemps, J.-D., 2012. Significant  
575 differences and curvilinearity in the self-thinning relationships of 11 temperate tree species  
576 assessed from forest inventory data. *Ann. For. Sci.* 69, 195–205.  
577 <https://doi.org/10.1007/s13595-011-0149-0>

578 Christiansen, E., Waring, R.H., Berryman, A.A., 1987. Resistance of conifers to bark beetle  
579 attack: Searching for general relationships. *For. Ecol. Manag.* 22, 89–106.  
580 [https://doi.org/10.1016/0378-1127\(87\)90098-3](https://doi.org/10.1016/0378-1127(87)90098-3)

581 Davi, H., Cailleret, M., 2017. Assessing drought-driven mortality trees with physiological  
582 process-based models. *Agric. For. Meteorol.* 232, 279–290.  
583 <https://doi.org/10.1016/j.agrformet.2016.08.019>

584 David-Schwartz, R., Paudel, I., Mizrachi, M., Delzon, S., Cochard, H., Lukyanov, V., Badel,  
585 E., Capdeville, G., Shklar, G., Cohen, S., 2016. Indirect Evidence for Genetic Differentiation  
586 in Vulnerability to Embolism in *Pinus halepensis*. *Front. Plant Sci.* 7.  
587 <https://doi.org/10.3389/fpls.2016.00768>

588 DELZON, S., DOUTHE, C., SALA, A., COCHARD, H., 2010. Mechanism of water-stress  
589 induced cavitation in conifers: bordered pit structure and function support the hypothesis of  
590 seal capillary-seeding. *Plant Cell Environ.* 33, 2101–2111. <https://doi.org/10.1111/j.1365-3040.2010.02208.x>

592 Diggle, P.J., 2013. Statistical analysis of spatial and spatio-temporal point patterns, 3rd ed. ed,  
593 Chapman & Hall/CRC Monographs on Statistics & Applied Probability. CRC Press,  
594 Hoboken, NJ.

595 Dubrovský, M., Hayes, M., Duce, P., Trnka, M., Svoboda, M., Zara, P., 2014. Multi-GCM  
596 projections of future drought and climate variability indicators for the Mediterranean region.  
597 *Reg. Environ. Change* 14, 1907–1919. <https://doi.org/10.1007/s10113-013-0562-z>

598 Ducrey, M., Duhoux, F., Huc, R., Rigolot, E., 1996. The ecophysiological and growth  
599 responses of Aleppo pine (*Pinus halepensis*) to controlled heating applied to the base of the  
600 trunk. *Can. J. For. Res.* 26, 1366–1374. <https://doi.org/10.1139/x26-152>

601 Durand-Gillmann, M., Cailleret, M., Boivin, T., Nageleisen, L.-M., Davi, H., n.d. Individual  
602 vulnerability factors of Silver fir (*Abies alba* Mill.) to parasitism by two contrasting biotic  
603 agents: mistletoe (*Viscum album* L. ssp. *abietis*) and bark beetles (Coleoptera: Curculionidae:  
604 Scolytinae) during a decline process. *Ann. For. Sci.* 1–15. <https://doi.org/10.1007/s13595-012-0251-y>

606 Ennajeh, M., Nouiri, M., Khemira, H., Cochard, H., 2011. Improvement to the air-injection  
607 technique to estimate xylem vulnerability to cavitation. *Trees* 25, 705–710.  
608 <https://doi.org/10.1007/s00468-011-0548-8>

609 Faccoli, M., Anfora, G., Tasin, M., 2008. Responses of the Mediterranean Pine Shoot Beetle  
610 *Tomicus destruens* (Wollaston) to Pine Shoot and Bark Volatiles. *J. Chem. Ecol.* 34, 1162–  
611 1169. <https://doi.org/10.1007/s10886-008-9503-6>

612 Ferrenberg, S., Kane, J.M., Mitton, J.B., 2014. Resin duct characteristics associated with tree  
613 resistance to bark beetles across lodgepole and limber pines. *Oecologia* 174, 1283–1292.  
614 <https://doi.org/10.1007/s00442-013-2841-2>

615 Fettig, C.J., Hilszczański, J., 2015. Chapter 14 - Management Strategies for Bark Beetles in  
616 Conifer Forests, in: Vega, F.E., Hofstetter, R.W. (Eds.), *Bark Beetles*. Academic Press, San  
617 Diego, pp. 555–584. <https://doi.org/10.1016/B978-0-12-417156-5.00014-9>

618 Forrester, D.I., Tachauer, I.H.H., Annighofer, P., Barbeito, I., Pretzsch, H., Ruiz-Peinado, R.,  
619 Stark, H., Vacchiano, G., Zlatanov, T., Chakraborty, T., Saha, S., Sileshi, G.W., 2017.  
620 Generalized biomass and leaf area allometric equations for European tree species  
621 incorporating stand structure, tree age and climate. *For. Ecol. Manag.* 396, 160–175.  
622 <https://doi.org/10.1016/j.foreco.2017.04.011>

623 Fortin, M.-J., Dale, M.R.T., Hoef, J.M.V., 2016. Spatial Analysis in Ecology, in: Wiley  
624 StatsRef: Statistics Reference Online. American Cancer Society, pp. 1–13.  
625 <https://doi.org/10.1002/9781118445112.stat07766.pub2>

626 Franceschi, V.R., Krokene, P., Christiansen, E., Krekling, T., 2005. Anatomical and chemical  
627 defenses of conifer bark against bark beetles and other pests. *New Phytol.* 167, 353–376.

628 <https://doi.org/10.1111/j.1469-8137.2005.01436.x>  
 629 Galiano, L., Martínez-Vilalta, J., Lloret, F., 2011. Carbon reserves and canopy defoliation  
 630 determine the recovery of Scots pine 4 yr after a drought episode. *New Phytol.* 190, 750–759.  
 631 <https://doi.org/10.1111/j.1469-8137.2010.03628.x>  
 632 Gaylord, M.L., Kolb, T.E., Pockman, W.T., Plaut, J.A., Yepez, E.A., Macalady, A.K., Pangle,  
 633 R.E., McDowell, N.G., 2013. Drought predisposes piñon-juniper woodlands to insect attacks  
 634 and mortality. *New Phytol.* 198, 567–578. <https://doi.org/10.1111/nph.12174>  
 635 Girard, F., Vennetier, M., Ouarmim, S., Caraglio, Y., Misson, L., 2010. Polycyclism, a  
 636 fundamental tree growth process, decline with recent climate change: the example of *Pinus*  
 637 *halepensis* Mill. in Mediterranean France. *Trees* 25, 311–322. [https://doi.org/10.1007/s00468-](https://doi.org/10.1007/s00468-010-0507-9)  
 638 [010-0507-9](https://doi.org/10.1007/s00468-010-0507-9)  
 639 Godin, C., Caraglio, Y., 1998. A Multiscale Model of Plant Topological Structures. *J. Theor.*  
 640 *Biol.* 191, 1–46. <https://doi.org/10.1006/jtbi.1997.0561>  
 641 Grégoire, J.-C., Raffa, K.F., Lindgren, S.B.S., 2015. Economics and Politics of Bark Beetles.  
 642 Elsevier Inc. <https://doi.org/doi/10.1016/B978-0-12-417156-5.00015-0>  
 643 Griffon, S., de Coligny, F., 2014. AMAPstudio: An editing and simulation software suite for  
 644 plants architecture modelling. *Ecol. Model.*, Special Issue of the 4th International Symposium  
 645 on Plant Growth Modeling, Simulation, Visualization and Applications (PMA'12 ) 290, 3–10.  
 646 <https://doi.org/10.1016/j.ecolmodel.2013.10.037>  
 647 Haase, P., 1995. Spatial pattern analysis in ecology based on Ripley's K-function:  
 648 Introduction and methods of edge correction. *J. Veg. Sci.* 6, 575–582.  
 649 <https://doi.org/10.2307/3236356>  
 650 Harvey, B.J., Donato, D.C., Turner, M.G., 2014. Recent mountain pine beetle outbreaks,  
 651 wildfire severity, and postfire tree regeneration in the US Northern Rockies. *Proc. Natl. Acad.*  
 652 *Sci.* 111, 15120–15125. <https://doi.org/10.1073/pnas.1411346111>  
 653 Hicke, J.A., Allen, C.D., Desai, A.R., Dietze, M.C., Hall, R.J., Hogg, E.H., Kashian, D.M.,  
 654 Moore, D., Raffa, K.F., Sturrock, R.N., Vogelmann, J., 2012. Effects of biotic disturbances on  
 655 forest carbon cycling in the United States and Canada. *Glob. Change Biol.* 18, 7–34.  
 656 <https://doi.org/10.1111/j.1365-2486.2011.02543.x>  
 657 Kausrud, K., Økland, B., Skarpaas, O., Grégoire, J.-C., Erbilgin, N., Stenseth, N.C., 2012.  
 658 Population dynamics in changing environments: the case of an eruptive forest pest species.  
 659 *Biol. Rev.* 87, 34–51. <https://doi.org/10.1111/j.1469-185X.2011.00183.x>  
 660 Kleiber, C., Zeileis, A., 2019. AER: Applied Econometrics with R.  
 661 Klutsch, J.G., Negrón, J.F., Costello, S.L., Rhoades, C.C., West, D.R., Popp, J., Caissie, R.,  
 662 2009. Stand characteristics and downed woody debris accumulations associated with a  
 663 mountain pine beetle (*Dendroctonus ponderosae* Hopkins) outbreak in Colorado. *For. Ecol.*  
 664 *Manag.* 258, 641–649. <https://doi.org/10.1016/j.foreco.2009.04.034>  
 665 Kozłowski, T.T., Pallardy, S.G., 1996. Physiology of Woody Plants. Elsevier.  
 666 Krokene, P., 2015. Chapter 5 - Conifer Defense and Resistance to Bark Beetles, in: Vega,  
 667 F.E., Hofstetter, R.W. (Eds.), *Bark Beetles*. Academic Press, San Diego, pp. 177–207.  
 668 <https://doi.org/10.1016/B978-0-12-417156-5.00005-8>  
 669 Kurz, W.A., Dymond, C.C., Stinson, G., Rampley, G.J., Neilson, E.T., Carroll, A.L., Ebata,  
 670 T., Safranyik, L., 2008. Mountain pine beetle and forest carbon feedback to climate change.  
 671 *Nature* 452, 987–990. <https://doi.org/10.1038/nature06777>  
 672 Lieutier, F., Långström, B., Faccoli, M., 2015. Chapter 10 - The Genus *Tomicus*, in: Vega,  
 673 F.E., Hofstetter, R.W. (Eds.), *Bark Beetles*. Academic Press, San Diego, pp. 371–426.  
 674 <https://doi.org/10.1016/B978-0-12-417156-5.00010-1>  
 675 Lieutier, F., Mendel, Z., Faccoli, M., 2016. Bark Beetles of Mediterranean Conifers, in: Paine,  
 676 T.D., Lieutier, Francois (Eds.), *Insects and Diseases of Mediterranean Forest Systems*.  
 677 Springer International Publishing, Cham, pp. 105–197. <https://doi.org/10.1007/978-3-319->

678 24744-1\_6  
679 Manion, P.D., 1981. Tree disease concepts. xv + 399 pp.  
680 Marini, L., Ayres, M.P., Battisti, A., Faccoli, M., 2012. Climate affects severity and  
681 altitudinal distribution of outbreaks in an eruptive bark beetle. *Clim. Change* 115, 327–341.  
682 <https://doi.org/10.1007/s10584-012-0463-z>  
683 Martin, G.L., Ek, A.R., 1984. A Comparison of Competition Measures and Growth Models  
684 for Predicting Plantation Red Pine Diameter and Height Growth. *For. Sci.* 30, 731–743.  
685 <https://doi.org/10.1093/forestscience/30.3.731>  
686 Martínez-Vilalta, J., 2014. Carbon storage in trees: pathogens have their say. *Tree Physiol.*  
687 *tpu010*. <https://doi.org/10.1093/treephys/tpu010>  
688 Morris, J.L., Cottrell, S., Fettig, C.J., DeRose, R.J., Mattor, K.M., Carter, V.A., Clear, J.,  
689 Clement, J., Hansen, W.D., Hicke, J.A., Higuera, P.E., Seddon, A.W., Seppä, H., Sherriff,  
690 R.L., Stednick, J.D., Seybold, S.J., 2018. Bark beetles as agents of change in social–  
691 ecological systems. *Front. Ecol. Environ.* 16, S34–S43. <https://doi.org/10.1002/fee.1754>  
692 Netherer, S., Matthews, B., Katzensteiner, K., Blackwell, E., Henschke, P., Hietz, P.,  
693 Pennerstorfer, J., Rosner, S., Kikuta, S., Schume, H., Schopf, A., 2015. Do water-limiting  
694 conditions predispose Norway spruce to bark beetle attack? *New Phytol.* 205, 1128–1141.  
695 <https://doi.org/10.1111/nph.13166>  
696 Paine, T.D., Raffa, K.F., Harrington, T.C., 1997. Interactions among scolytid bark beetles,  
697 their associated fungi, and live host conifers. *Annu. Rev. Entomol.* 42, 179–206.  
698 <https://doi.org/10.1146/annurev.ento.42.1.179>  
699 Peverieri, G.S., Capretti, P., Tiberi, R., 2006. Associations between *Tomicus destruens* and  
700 *Leptographium* spp. in *Pinus pinea* and *P. pinaster* stands in Tuscany, central Italy. *For.*  
701 *Pathol.* 36, 14–20. <https://doi.org/10.1111/j.1439-0329.2006.00427.x>  
702 Pitman, G.B., 1969. Pheromone Response in Pine Bark Beetles: Influence of Host Volatiles.  
703 *Science* 166, 905–906. <https://doi.org/10.1126/science.166.3907.905>  
704 Pringle, R.M., Doak, D.F., Brody, A.K., Jocqué, R., Palmer, T.M., 2010. Spatial Pattern  
705 Enhances Ecosystem Functioning in an African Savanna. *PLOS Biol.* 8, e1000377.  
706 <https://doi.org/10.1371/journal.pbio.1000377>  
707 Raffa, K.F., Aukema, B.H., Bentz, B.J., Carroll, A.L., Hicke, J.A., Turner, M.G., Romme,  
708 W.H., 2008. Cross-scale Drivers of Natural Disturbances Prone to Anthropogenic  
709 Amplification: The Dynamics of Bark Beetle Eruptions. *BioScience* 58, 501–517.  
710 <https://doi.org/10.1641/B580607>  
711 Raffa, K.F., Aukema, B.H., Erbilgin, N., Klepzig, K.D., Wallin, K.F., 2005. Interactions  
712 among conifer terpenoids and bark beetles across multiple levels of scale: An attempt to  
713 understand links between population patterns and physiological processes. *Recent Adv.*  
714 *Phytochem.* 39 79-118.  
715 Raffa, K.F., Berryman, A.A., 1982. Physiological Differences Between Lodgepole Pines  
716 Resistant and Susceptible to the Mountain Pine Beetle and Associated Microorganisms.  
717 *Environ. Entomol.* 11, 486–492. <https://doi.org/10.1093/ee/11.2.486>  
718 Raffa, K.F., Grégoire, J.-C., Staffan Lindgren, B., 2015. Chapter 1 - Natural History and  
719 Ecology of Bark Beetles, in: Vega, F.E., Hofstetter, R.W. (Eds.), *Bark Beetles*. Academic  
720 Press, San Diego, pp. 1–40. <https://doi.org/10.1016/B978-0-12-417156-5.00001-0>  
721 Ripley, B., Venables, W., 2016. *nnet: Feed-Forward Neural Networks and Multinomial Log-*  
722 *Linear Models*.  
723 Ripley, B.D., 1976. The Second-Order Analysis of Stationary Point Processes. *J. Appl.*  
724 *Probab.* 13, 255–266. <https://doi.org/10.2307/3212829>  
725 Salomón, R.L., Limousin, J.-M., Ourcival, J.-M., Rodríguez-Calcerrada, J., Steppe, K., 2017.  
726 Stem hydraulic capacitance decreases with drought stress: implications for modelling tree  
727 hydraulics in the Mediterranean oak *Quercus ilex*: Seasonality in stem hydraulic capacitance.

728 Plant Cell Environ. <https://doi.org/10.1111/pce.12928>  
729 Sauvard, D., Lieutier, F., Levieux, J., 1987. Répartition spatiale et dispersion de *Tomicus*  
730 *piniperda* L.(Coleoptera Scolytidae) en forêt d'Orléans, in: *Annales Des Sciences Forestières*.  
731 EDP Sciences, pp. 417–434.  
732 Scholander, P.F., Bradstreet, E.D., Hemmingsen, E.A., Hammel, H.T., 1965. Sap Pressure in  
733 Vascular Plants: Negative hydrostatic pressure can be measured in plants. *Science* 148, 339–  
734 346. <https://doi.org/10.1126/science.148.3668.339>  
735 Weed, A.S., Ayres, M.P., Hicke, J.A., 2013. Consequences of climate change for biotic  
736 disturbances in North American forests. *Ecol. Monogr.* 83, 441–470.  
737 <https://doi.org/10.1890/13-0160.1>  
738

739

740

741

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
<b>Predisposing factors</b>	DBH <sub>2011</sub>	DBH <sub>2011</sub>	$\Psi_{50}$	$\Psi_{50}$
	Height <sub>2011</sub>	Height <sub>2011</sub>		
	Competition	Competition		
	Leaf Color <sub>2011</sub>	Leaf Color <sub>2011</sub>		
	Defoliation <sub>2011</sub>	Defoliation <sub>2011</sub>		
	RW <sub>1950-2010</sub>	RW <sub>1950-2010</sub>		
	WD <sub>950-2010</sub>	WD <sub>1950-2010</sub>		
<b>Effect variables</b>	Leaf Color <sub>2012</sub>	Leaf Color <sub>2012</sub>	$\Psi_{50}$	$\Psi_{50}$
	Defoliation <sub>2012</sub>	Defoliation <sub>2012</sub>	Weekly growth	Weekly
	RW <sub>2011-2012</sub>	RW <sub>2011-2012</sub>	Sugars	growth
	$\rho_{\text{wood}2011-2012}$	$\rho_{\text{wood}2011-2012}$		

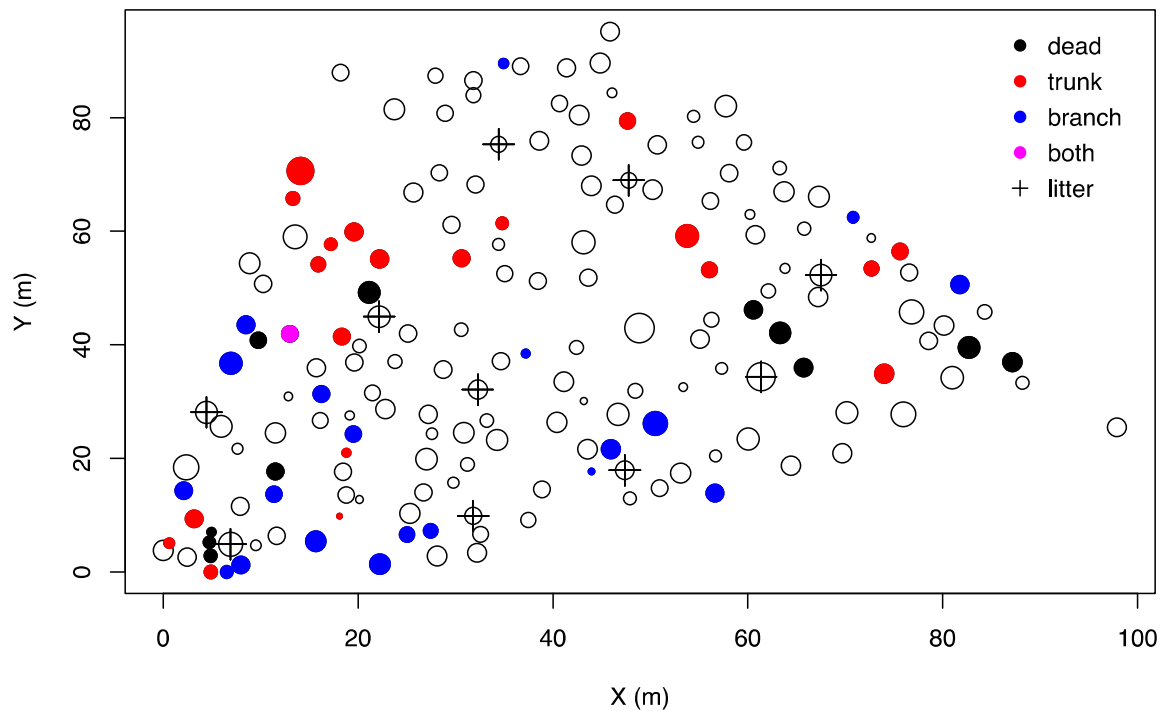
745

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

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

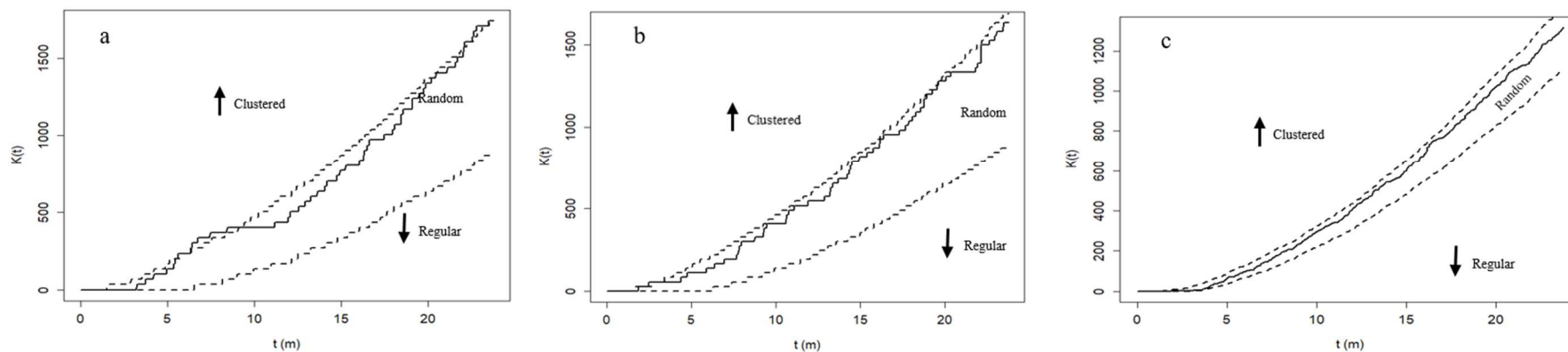
751





753  
 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.

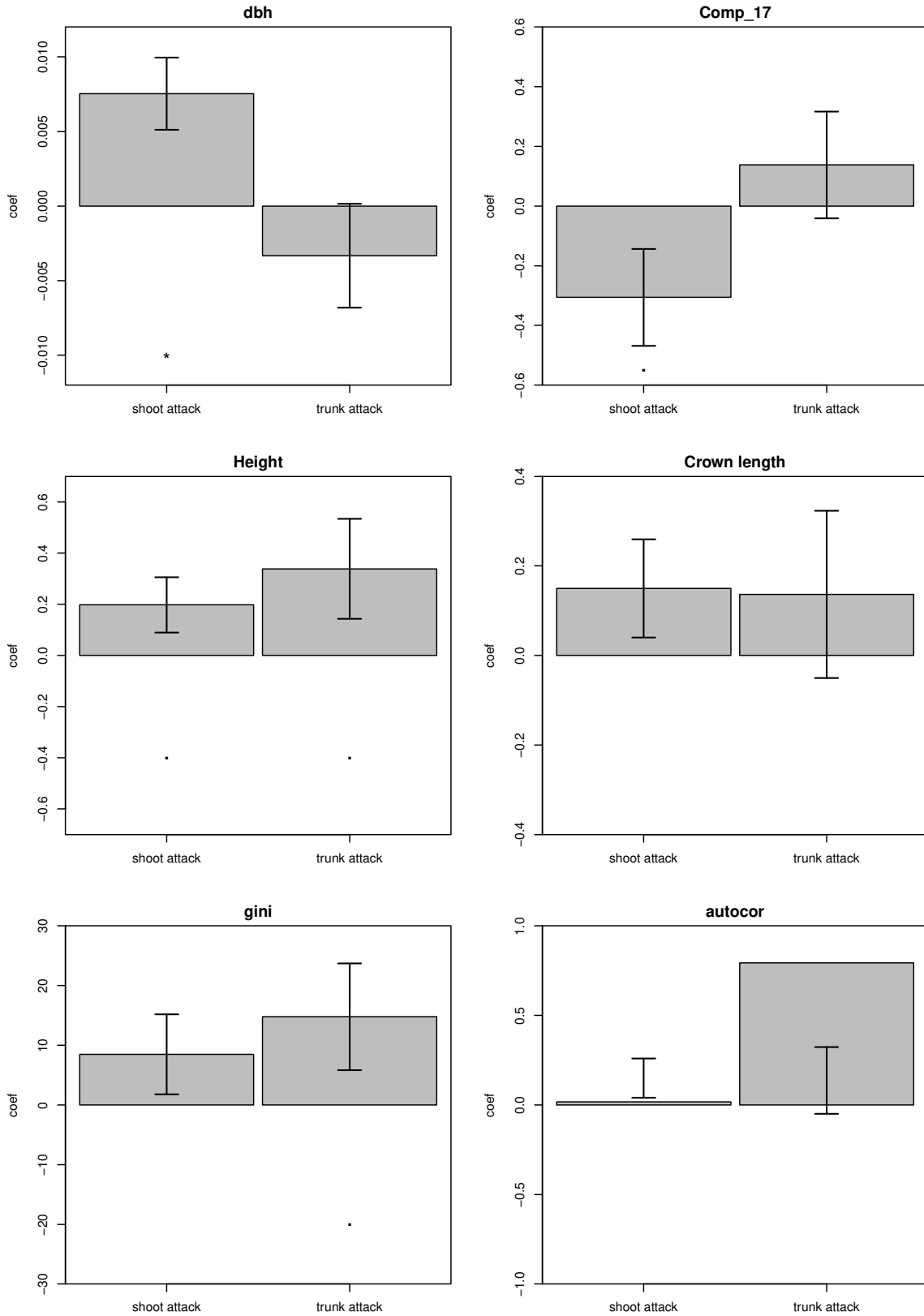
760



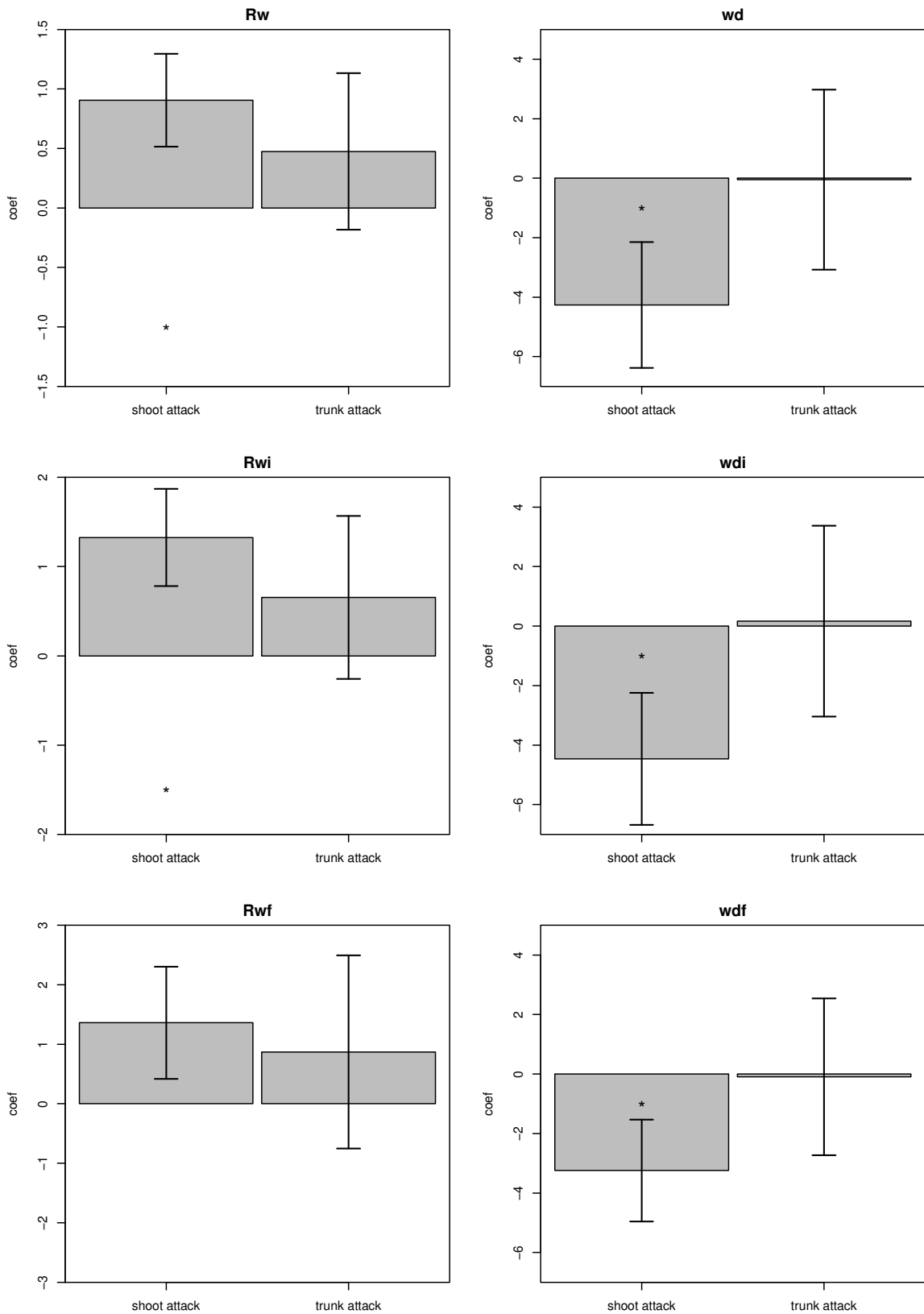
761

762

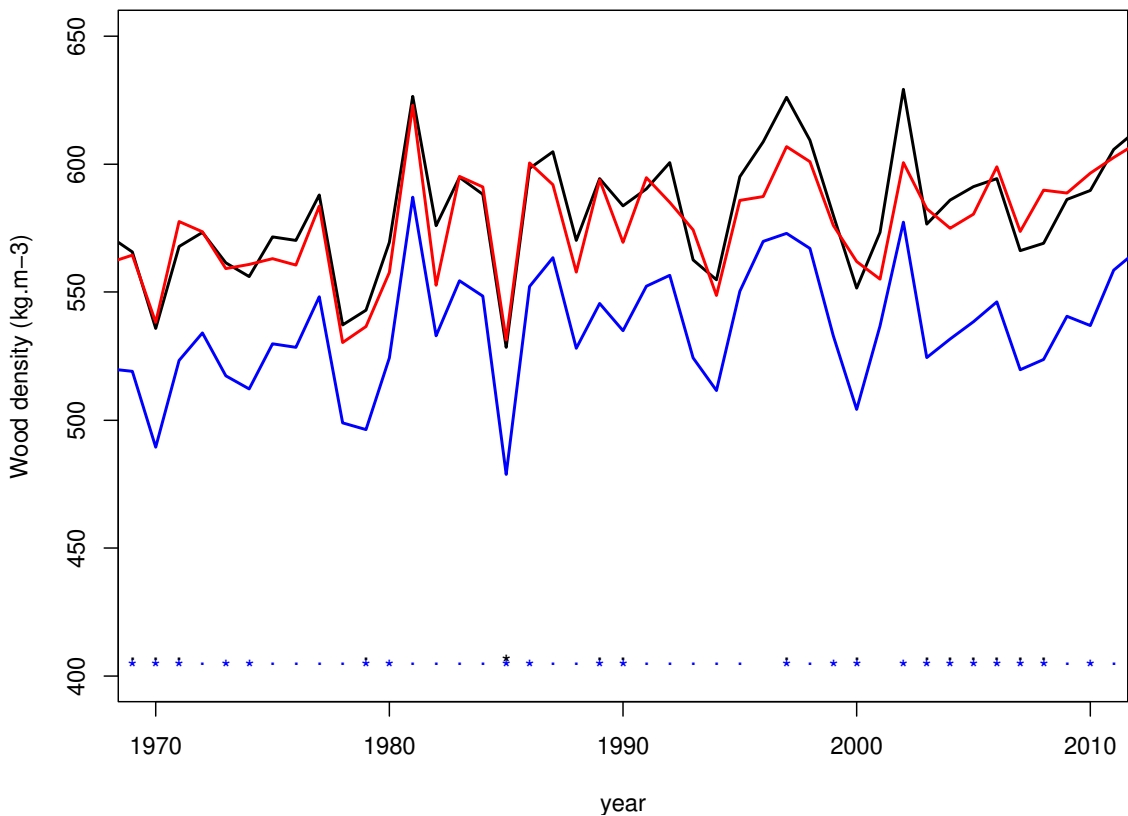
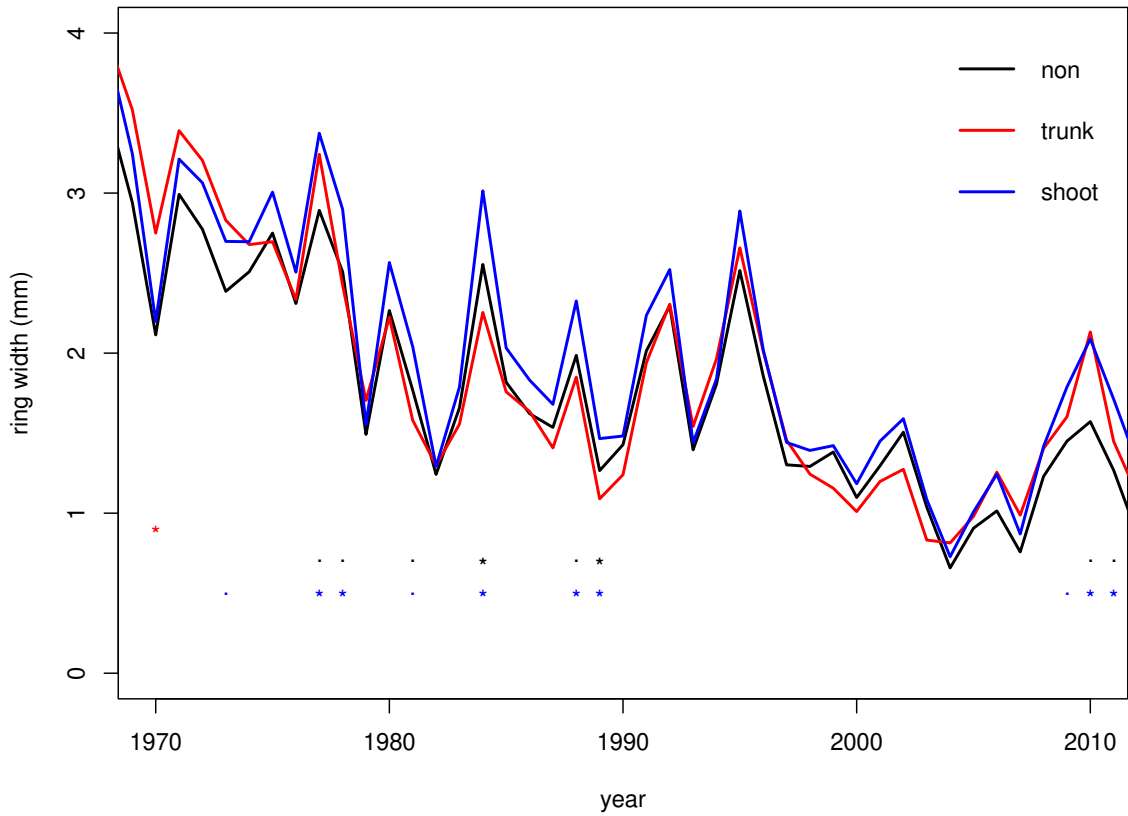
763 **Figure 2.** Univariate spatial pattern analysis using the Ripley's K-function for distributions of *Tomicus destruens* attacks in a *Pinus halepensis*  
764 population in Southern France. The figure shows the observed statistic  $K(t)$  for trunk attacks for the insect's breeding phase in fall 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.



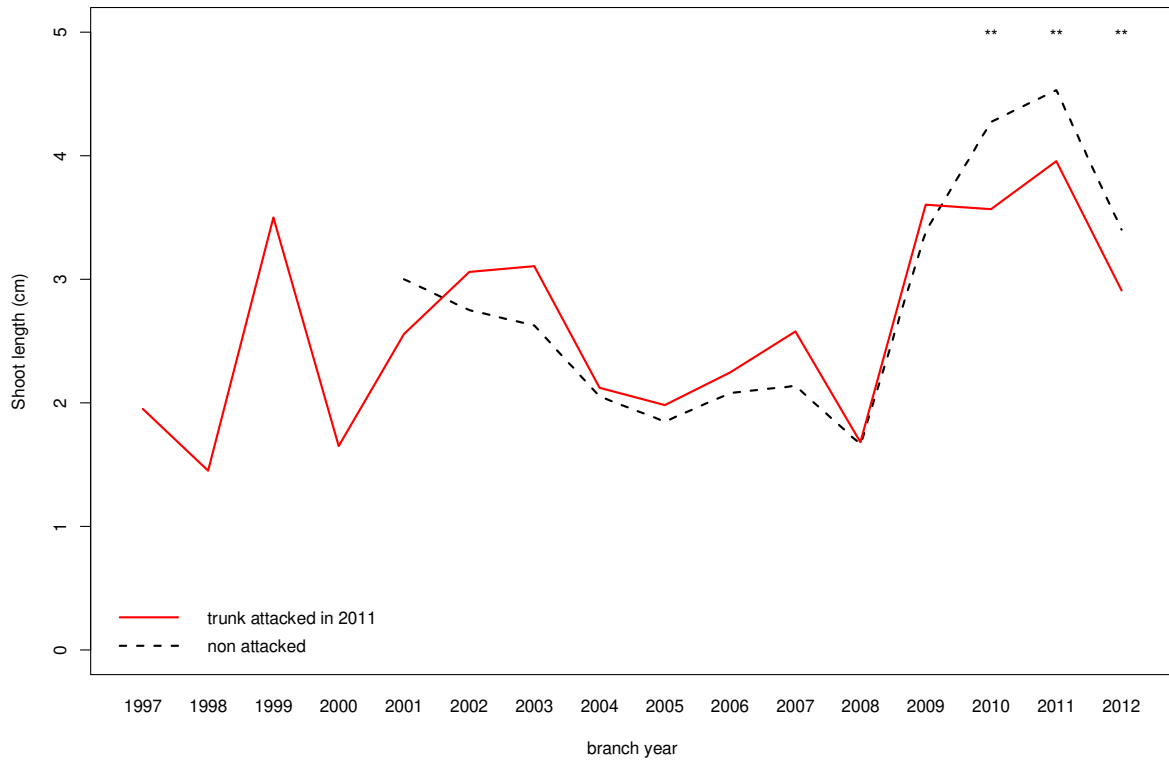
770 **Figure 3a:** Dendrometric factors predisposing *Tomicus destruens* attacks in a *Pinus*  
 771 *halepensis* population in Southern France. Coefficient of the logistic regression analysis, non-  
 772 attacked trees being taken as a reference. A star indicates that the coefficient is significantly  
 773 different from the reference with a p-value < 0.05 and a point with a p-value < 0.1.  
 774



775  
 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.

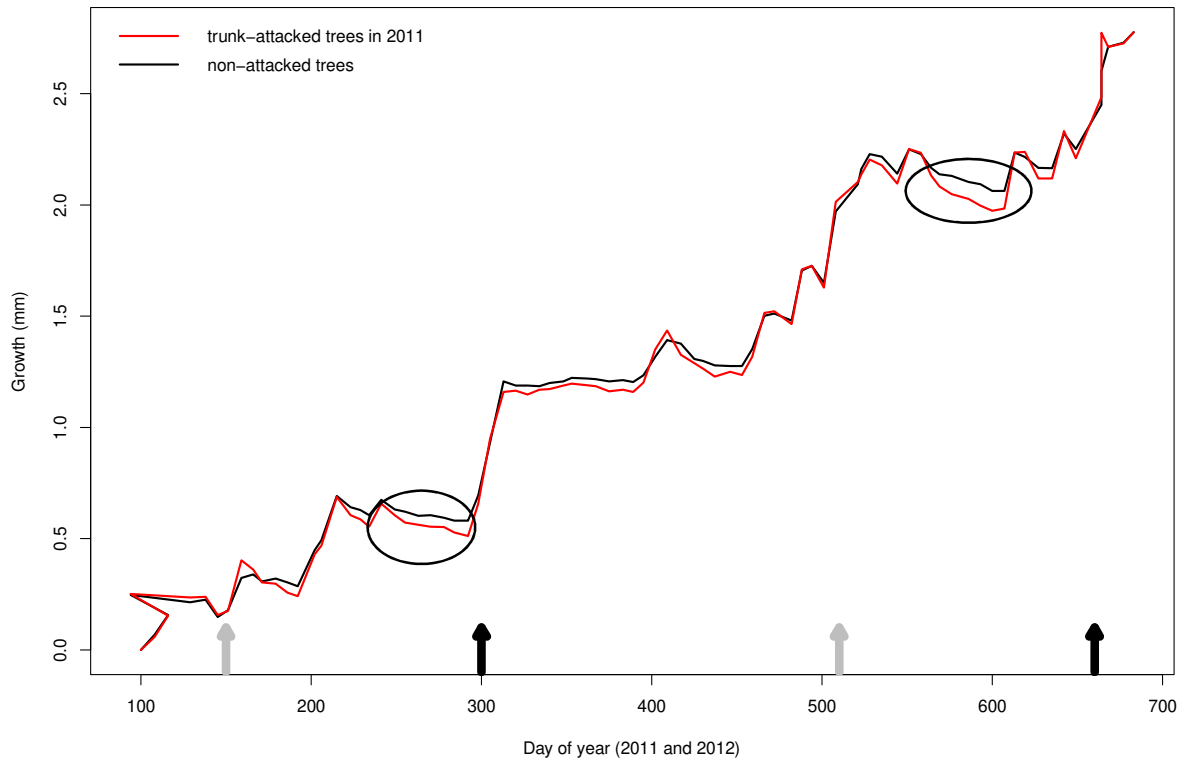


780  
 781 **Figure 4:** Past growth (ring width in mm) and wood density of non-attacked (black), shoot  
 782 attacked (red) and trunk attacked (blue) individuals of *Pinus halepensis* in southeastern  
 783 France. Significant differences between trunk attacked trees and non-attacked trees in red (\*  
 784 at 5% and at 10%). Significant differences between trunk attacked trees and non-attacked  
 785 trees in blue. Whole significant differences in black



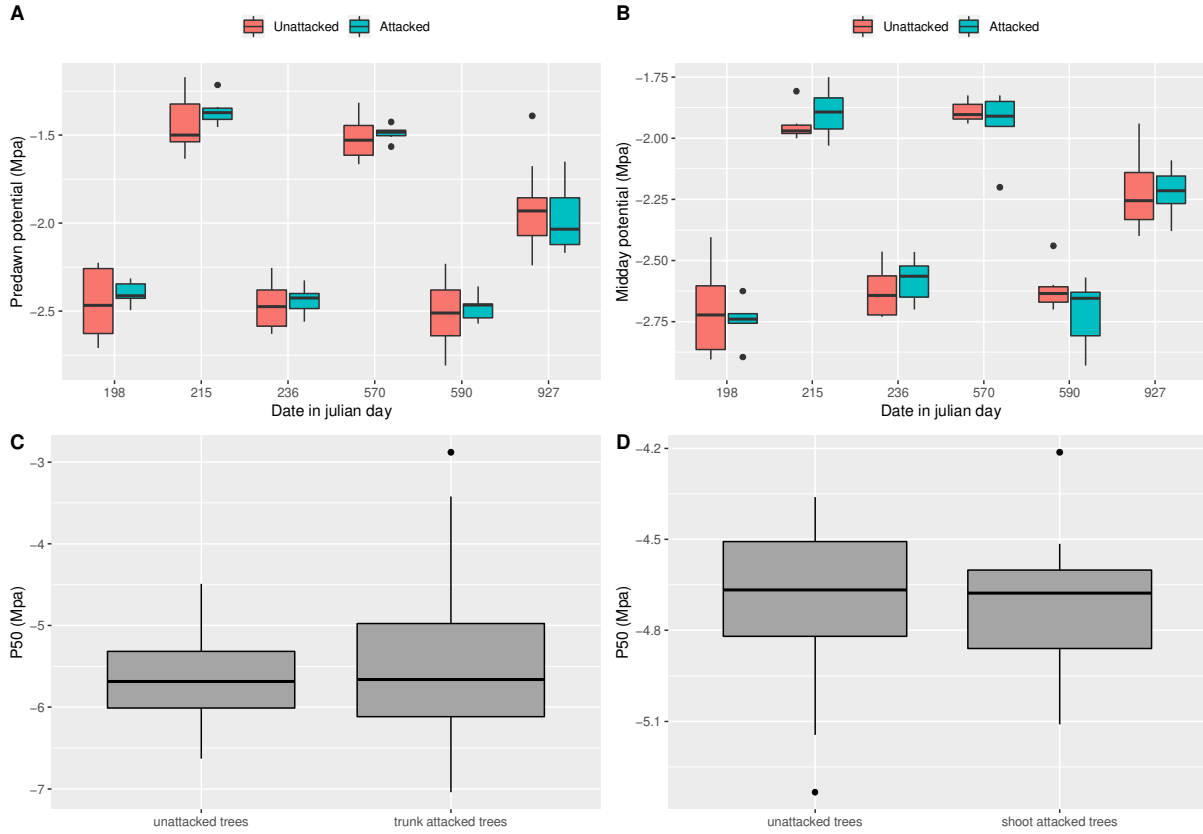
787  
788  
789  
790

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



792  
 793  
 794  
 795  
 796  
 797

**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.



799

800

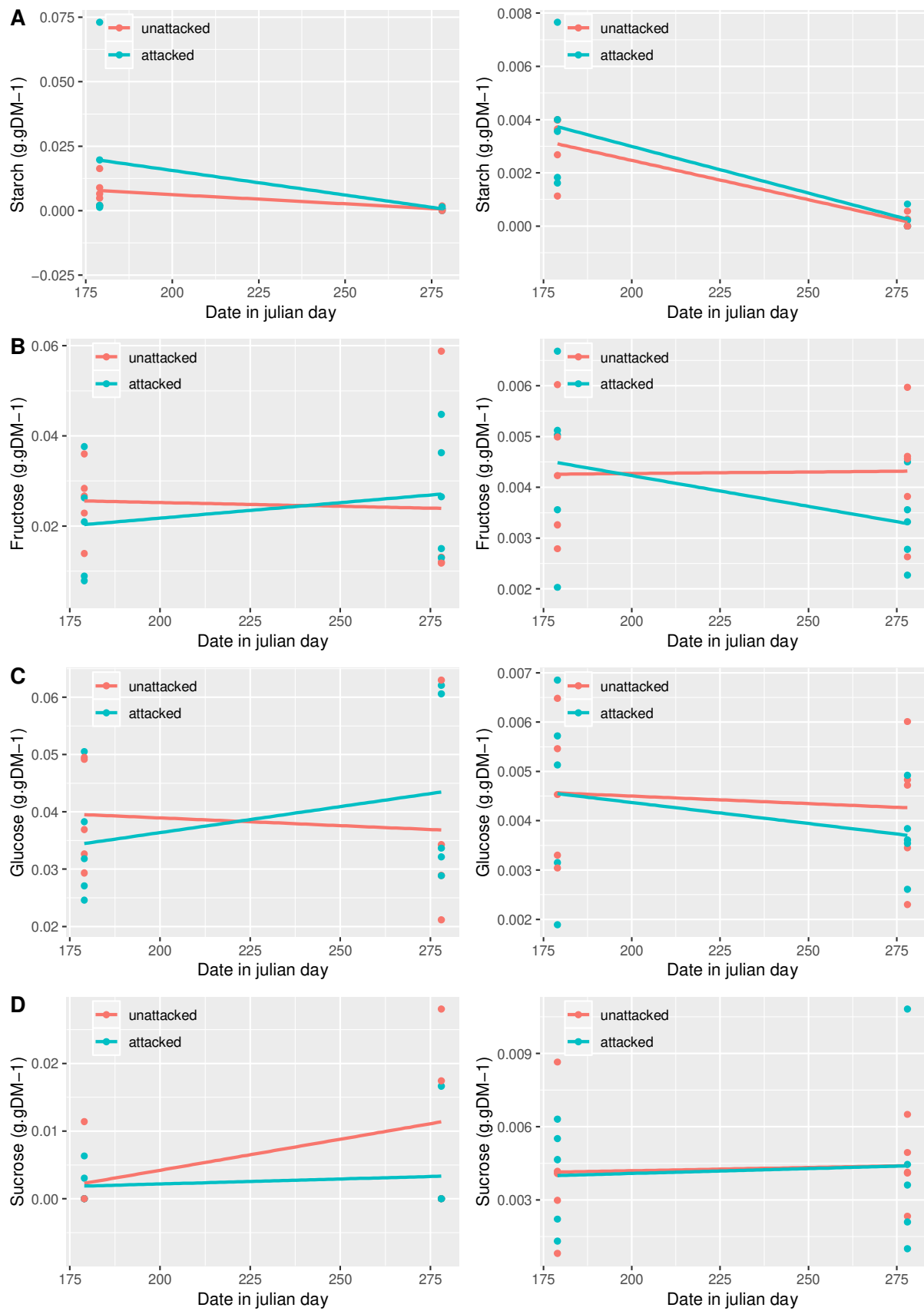
801

802

803

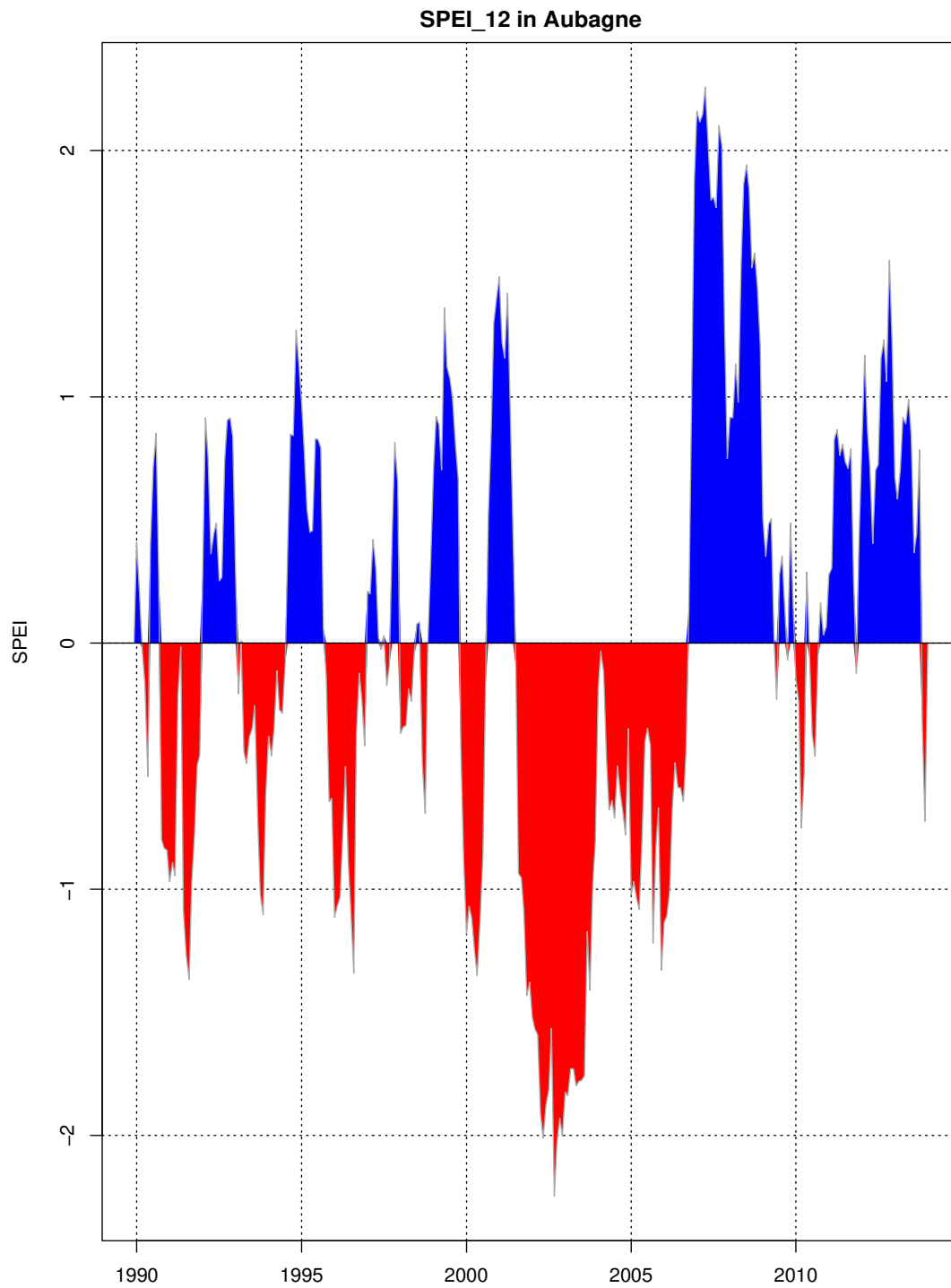
**Figure 7:** (a, b) Effect of 2011 trunk attacks on predawn and midday water potentials dynamics (Mpa) on *Pinus Halepensis* trees. (c, d) Effect of trunk attacks (in 2011) and shoot attacks (in 2012) by *Tomicus destruens* on *Pinus Halepensis* xylem water potential inducing 50% of conductivity (P50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





804  
805  
806  
807  
808

**Figure 8: Non structural carbohydrates concentration ( $\text{g g}_{\text{DM}}^{-1}$ , 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).**

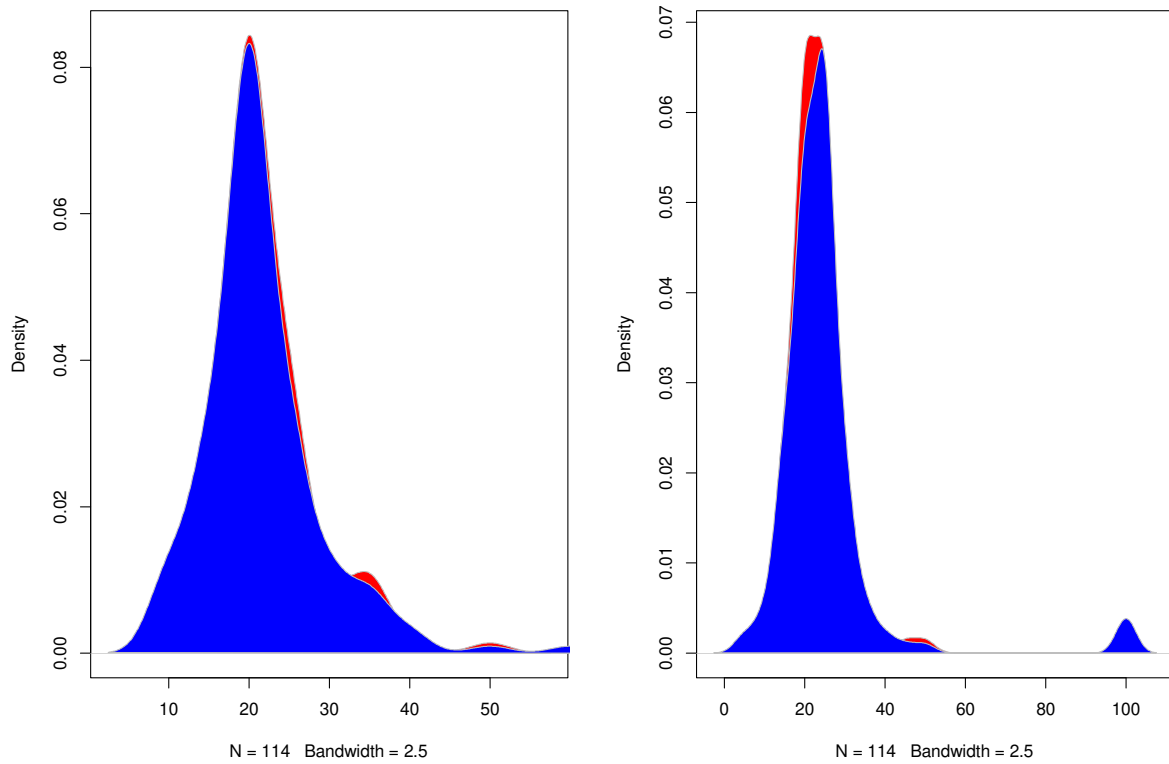


810

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.

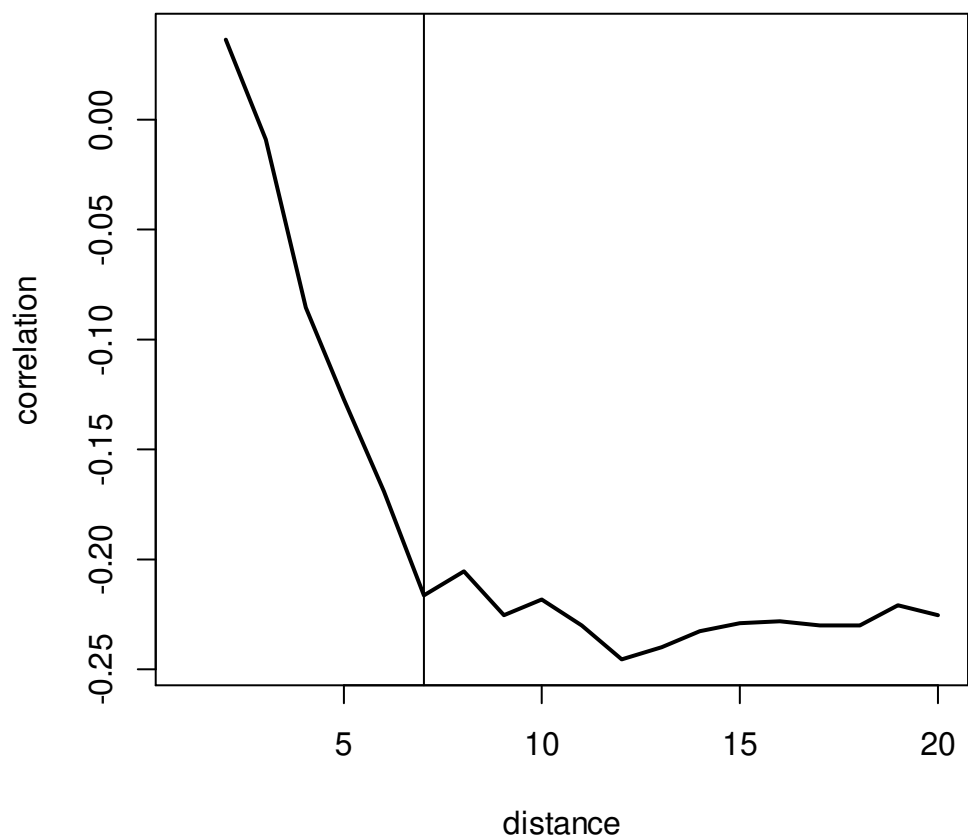
814

815

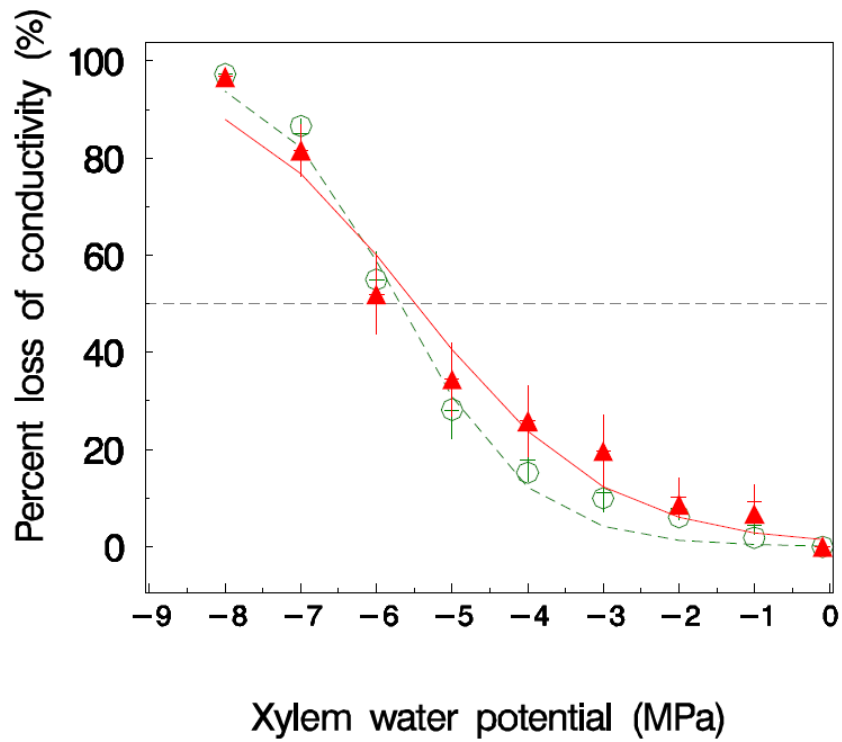


816  
 817  
 818  
 819  
 820  
 821  
 822

**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).



823  
 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.  
 827



828  
829  
830  
831  
832

**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.