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

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Abstract

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

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

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

Introduction

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

Dispersing adult bark beetles colonize their host tree by boring an entrance hole through the bark. Females dig a tunnel near the cambium in which they lay their eggs; developing broods dig their own tunnel by feeding on the phloem. In conifers, tree defense mechanisms against bark beetle attacks comprise a system of toxic resin ducts of variable size and number, and the synthesis of defensive chemicals at the entrance site (Ferrenberg et al., 2014). The resin, in addition to being toxic, presents a physical barrier that the insect needs to overcome. Thus, the likelihood of a tree's survival following a bark beetle attack is closely linked to the interplay between a tree's ability to mobilize its defense mechanisms and the profusion of attacking bark beetles (Paine et al., 1997). Consequently, stress factors that weaken a tree's defenses are usually associated with the initiation of bark beetle epidemics (Marini et al., 2012). Drought is particularly likely to increase a tree's susceptibility to insect attacks and to induce changes in its physiology (Gaylord et al., 2013; Netherer et al., 2015). Bark beetles are inherently labile forest insect populations displaying transitions between endemic states in which they reside in stands at very low densities and are expected to kill only a few weakened trees, and epidemic states in which they reach very high densities over large areas causing high tree mortality at both the stand and landscape scales (Kausrud et al., 2012). Knowledge on the impacts of insect epidemics on tree individuals and populations has benefitted from extensive research (Raffa et al., 2008; Grégoire et al., 2015), although less attention has been paid to those associated with endemic states (Raffa et al., 2005; Boone et al., 2011; Bleiker et al., 2014).

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

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

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

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

Materiel and Methods

Tree-insect system

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

The pine shoot beetle, *T. destruens,* is primarily attracted by volatiles emitted from fresh underbarked timber and dying or stressed pines. It takes advantage of favorable climatic conditions and increased resource availability to attack healthy trees (Branco et al., 2010; Lieutier et al., 2016). *T. destruens* is one of the few bark beetle species to display a continuous life cycle throughout the year (Lieutier et al., 2016). This species is characterized by two dispersal phases during its univoltine life cycle. Sexually immature adults emerge in spring (April-May) from one to two larval cohorts (i.e. sister broods) that have developed in the trunks over winter before flying towards the crowns of healthy pines and tunneling into vigorous newer green shoots to complete sexual maturation. In late autumn (October-November), sexually mature adults of *T. destruens* leave the shoots to seek recently cut trees on which to mate and lay their eggs under the bark (Faccoli et al., 2008). The larvae feed and develop in the phloem while pupation occurs in the outer bark. Adults carry spores of associated fungal species (e.g. *Leptographium* spp.) that spread in the phloem and contribute to wood devaluation through wood color alteration (Bezos et al., 2015; Bois and Lieutier, 1997; Peverieri et al., 2006). The presence of *T. destruens* is essentially indicated by pitch tubes of resin, sawdust produced by insect penetration on the trunks and green or red shoots, and a medullary gallery on the ground.

Study site

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

Spatial distributions of insect attacks

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

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

For each tree (n = 178), we measured the tree height, tree diameter, level of competition, past growth using ring width, ring wood density, tree health status, and the presence of bark beetle attacks on the trunk and/or shoots. On a sub-sample of trees (n =12), we also assessed the weekly diameter growth, leaf surface, Huber Value at the branch level (i.e. the ratio between the leaf area and the stem diameter supporting the leaves), the Non-Structural Carbohydrate (NSC) content in xylem and phloem, the leaf water potential (pre-dawn and midday), and the vulnerability to embolism. According to its temporal positioning with respect to a bark beetle attack, the measured physiological characteristic is either a predisposing factor to the attack or a physiological consequence of this attack (Table 1). These twelve trees had similar diameters (range: 25.5 - 38.2 cm); six were healthy and six had attacked trunks.

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

$$\mathrm{CI}_{\mathrm{MAEK,i}} = \frac{1}{\mathrm{dbh}_i}\sum_{\mathrm{j}=1}^{n}\left(\mathrm{dbh}_i * \exp\left[\frac{-16 * d_{\mathrm{ij}}}{\mathrm{dbh}_i+\mathrm{dbh}_j}\right]\right)$$

Past radial growth and wood density were assessed using micro-densitometric and ring-width profiles. One core per tree was taken in winter 2012-2013 using an increment borer at breast height (DBH; 1.30 m); this was performed perpendicular to the slope to avoid sampling bias. To estimate the trees' ring widths, cores were taken using a razor blade and then digitalized with a high-resolution scanner. Ring width (RW; millimeters per year) chronologies were measured at a precision of 0.01 mm and were cross-dated using CDendro v5.3 and CooRecorder 5.3 software (Cybis Elektronik & Data AB, Sweden). For each tree, the ring-width chronologies were obtained and cross-dated according to a reference chronology in order to delimit missing or false rings, and thus standardize the radial growth data by removing the age effect. To estimate the wood density, two-millimeter segments were taken from the cores using twin saws, and the resin from each core was removed with pentane. The samples were X-rayed and the resulting radiographic films were digitized with a high-resolution scanner after resin removal. Density levels were associated with the gray levels in the image by means of a previously established calibration curve. Windendro® software (Regent Instruments Inc., Canada) was used to delimit the annual growth ring and calculate the micro-densitometric profiles. Only 115 trees were retained in the end as some cores were damaged during sawing, and some images were blurred during analysis. The sub-sample of cored trees shows a similar distribution of defoliation levels to the whole sample (Figure s2). A detrend analysis was performed using a spline function (DplR package in R) to remove age effects. Common statistics were estimated on raw ring width series (rw: ring with, rwi: early wood width, rwf: late wood width, wd: wood density, wdi: wood density of early wood, wdf: wood density of late wood) and detrended ring widths (autoCorr: ring width autocorrelation and Gini).

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

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

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

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

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

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

Finally, leaf water potential and xylem vulnerability to embolism were assessed. Pre-dawn leaf water potential was measured in 2011, 2012 and 2013 in summer before sunrise. Two shoots per tree, with a stem of 2 cm minimum, were collected. The samples were placed in individual bags in the dark and then put into a chiller to maintain their inner branch pressure (avoiding all contact with ice). Leaf water potential was measured with a Scholander bomb or a pressure chamber (Scholander et al., 1965). In the laboratory (and within one hour of collection), vulnerability to embolism was determined through vulnerability curves obtained in 2013 and 2014 on trees surveyed for leaf water potential, leaf traits and weekly growth, and on 24 shoot-attacked and healthy trees respectively. Vulnerability curves and xylem-specific hydraulic conductivity were assessed using an air-injection method in Avignon for the first sample in 2013, and with the CAVITRON at INRA Bordeaux for the second in 2014 (see Delzon et al., 2010 for methodological details). In previous studies, P50 was found to be the same using both techniques (Ennajeh et al., 2011). Stems were wrapped in moist paper and placed in plastic bags to avoid water loss through transpiration. They were immediately sent to the CAVIPLACE laboratory where they were stored at 4 °C prior to measurements (two weeks maximum in this case). For the measurements, the shoots were debarked and cut under water to a standard length of 27 cm; both ends were pared with a razor blade. Samples were not flushed with water in order to avoid possible effects of air-seeding fatigue due to stretching or degrading the pit membranes during the previous embolism events. A solution of ultra-pure deionized water containing 10mM KCl and 1mM CaCl 2 was used as the reference ionic solution. Hydraulic conductivities at every rotation speed were measured using Cavisoft software (v. 5.2, University of Bordeaux) in order to obtain vulnerability curves corresponding to the percentage loss of hydraulic conductivity as a function of xylem pressure.

Statistical analysis

Spatial patterns of trunk and shoot attacks by *T. destruens* were inferred from a multi-distance spatial cluster analysis with Ripley’s K-function (Ripley, 1976), which has been widely applied to distribution patterns of herbs, forest trees and sedentary animals (Fortin et al., 2016; Haase, 1995; Pringle et al., 2010). We used Ripley's method for the univariate point pattern analysis in a two-dimensional space based on the distances between tree individuals to test whether a process (i.e. trunk or shoot attacks) was clustered at one or multiple different distances within the pine plot. Observed repartitions of attacked trees were compared to simulated repartitions obtained by randomly distributing the same number of attacks among trees in the stand. Spatial patterns of insect attacks were inferred by defining both upper and lower 95% confidence thresholds for the observed statistic $K(t)$ of each process of attack at the inter-individual distance t based on 1,000 simulations of the process. Spatial distributions of insect attacks are considered as being: clustered when $K(t)$ is above the upper confidence limit, having complete spatial randomness when $K(t)$ is within the 95% confidence threshold, and regular when $K(t)$ falls below the lower confidence threshold (Diggle, 2013; Fortin et al., 2016). These analyses were performed with the spatstat package in R (Baddeley et al., 2011).

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

$$Class_{tree} = Factor_{tree} + \epsilon_{tree}$$

Tests on the sub-samples of the initial population were carried out using rank statistics (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^{-1}) with a Relative Density Index of 0.5 when using the equations of Charru et al., (2012). Consequently, competition explained only 6% of the growth variation between trees suggesting this competition was probably driven more by light than water as the correlation between growth and competition no longer increased beyond a seven-meter radius (Figure s3).

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

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

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

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

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

Tree diameter is not linked to water potential and vulnerability to embolism. Therefore, it has not been taken into account as a co-variable in the following analyzes. The shoots sampled for vulnerability curve measurements were sufficiently long (> 15 cm) to represent more than five years of growth. Vulnerability to embolism, measured in 2013 and 2014 for 2011 trunk-attacked trees and in 2012 for shoot-attacked trees, may be considered both as a predisposing factor for an attack and as a consequence of an attack. The P50 values ranged from -7.04 MPa to -2.88 Mpa with an average value of -4.85 Mpa. Neither the trees attacked on the shoots in 2012 (p = 0.6475) nor the trees attacked on the trunks in 2011 (p = 0.8798) showed significant differences in the xylem vulnerability to embolism (P50, pressure inducing 50% loss of conductivity loss) compared to non-attacked trees (Figure 7a). Although the P50 values were similar, the trunk-attacked trees appeared to be slightly more vulnerable to embolism at the onset of water stress (Figure s4). Conductivity (Ksmax) was not affected by trunk attacks (p = 0.16) or by shoot attacks (p = 0.47). In addition, neither the pre-dawn water potential (p = 0.872) nor the midday water potential (p = 0.9) of trunk-attacked trees differed from those of unaffected trees (Figure 7b). Some of the trees whose water potential was surveyed from 2011, were also attacked on their shoots in 2012. There was no effect of attacks on shoots in 2012 nor on the midday potential (p = 0.757) or on the pre-dawn potential (p = 0.881).

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

Discussion

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

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

These results suggest that the distribution of *T. destruens* attacks in an endemic state were not a consequence of the distribution of the health status of the trees but rather the dispersal strategies of the bark beetles, which change during different phases in their life cycle. For bark beetles, one of the major limitations of fitness at low population density is the inability to successfully attack trunks of healthy trees exhibiting fully expressed resistance mechanisms. This implies a constraint on insects' foraging for the location of more poorly protected resources for breeding (Kausrud et al., 2012). The predominant strategy among most bark beetle groups is based on pheromone-mediated cooperative behavior to accumulate on a tree in order to overcome its defenses (Pitman, 1969; Raffa et al., 2015), although *T. destruens* is one exception (Lieutier et al., 2015). Trunk colonization by pine shoot beetles proceeds from repeated tree interceptions by pioneer beetles through random flights and by the subsequent increase in tree attractivity for new colonizers due to the host volatiles (e.g. α-pinene, terpinolene, ethanol) stimulated by the pioneer attacks (Byers, 1992; Lieutier et al., 2015). This is likely to generate aggregative patterns of individual trees subjected to trunk attacks if the attracted pioneer congeners fail to locate the target tree and land on surrounding ones. Our results for *T. destruens* were consistent with aggregative attacks of other pine species by the closely related species *T. piniperda* during its breeding phase (Lieutier et al., 2015; Sauvard et al., 1987).

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

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

The mean P50 values found in this study are very similar to the ones reported in different *P. halepensis* provenances growing in arid environments (David-Schwartz et al., 2016). The vascular system was unaffected by trunk attacks as suggested by the lack of differences in xylem vulnerability to embolism and water potential. Nevertheless, we found that trunk-attacked trees showed greater trunk shrinkage during drought episodes. This suggests that attacks can affect tree capacitance and thus their resistance to very pronounced droughts (Salomón et al., 2017). While trunk attacks did not affect secondary growth they did lead to a decrease in primary growth, as well as higher defoliation and foliage discoloration in the year following the attack; this generally reduced photosynthesis (Galiano et al., 2011).

When significant amounts of stored resin are exuded from the resin ducts in the phloem and xylem, the intruding beetles may be repelled or 'pitched out'. This resin is highly toxic to bark-beetle eggs and larvae and inhibits fungal growth. In lodgepole pines, a series of biochemical alterations are associated with insect attacks including a pronounced increase in monoterpene levels, the conversion of a monoterpene composition into more toxic and repellent compounds, and the accumulation of heartwood phenolics (Raffa and Berryman, 1982). Prior to and during the expression of active wound physiology, the metabolically passive flow of resin from severed ducts can delay beetle progression into the cambium thus giving the trees more time to respond to attacks by employing other chemical defense mechanisms (Raffa and Berryman, 1982). The oleoresin synthesized by pines is derived from isoprenoid units (isopentenyl diphosphate); these terpenes are mostly biosynthesized in the cambium zone through the classic mevalonic acid pathway from acetyl-CoA in cytoplasm, or through the MEP(2-C-Methyl-D-erythritol 4-phosphate) pathway in chloroplast. However, exudation of the constitutive resin depends on fructose and glucose availability within the duct system, and the viscosity of the oleoresin (Christiansen et al., 1987). This carbon-heavy defense mechanism can have implications for the tree’s carbon balance (Martínez-Vilalta, 2014). In this study, it might explain the decrease in fructose and glucose concentrations on attacked trees in the spring following the winter trunk attacks. Such lower levels of fructose and glucose could stimulate their production by local hydrolysis of the starch. This may be of benefit to insects as a feeding substrate (Raffa et al., 2015), support induced resinosis following insect attacks (Krokene, 2015), and participate in maintaining ring growth even if the phloem transport is locally disrupted. As the lateral movement of sugars between the xylem and the phloem have already been reported (Aubry et al., 2019), changes in fructose and glucose levels in the phloem can also induce changes in the xylem. Moreover, in the Pinaceae, the traumatic ducts are formed in the xylem (Franceschi et al., 2005), which may also explain the sucrose changes in xylem. Finally, as gymnosperm have high lipid content (Kozlowski and Pallardy, 1996), further quantifications of lipid reserve dynamics would be of critical interest in terms of examining their potential role as an insect food resource and in terms of growth sustainment.

Conclusions and perspectives

In this study, we show that a population of *T. destruens* at an endemic stage may not necessarily attack weakened host trees, and that notably they specifically targeted the shoots of the most vigorous trees during the sexual maturation phase of their life cycle. We also suggest that the aggregative nature of trunk attacks during the breeding phase may be due to insect foraging behavior in a context of rare suitable hosts, rather than depicting clusters of trees weakened by unfavorable micro-scale conditions (e.g. micro-edaphic). At such a low insect population level, attacks appeared to have little or no effect on tree health and we found a low tree mortality rate. In an endemic context, such weak effects of insect attacks support the strong resilience potential of trees which may have enough starch to face the physiological consequences of trunk attacks and that may not be affected by the subsequent falling of attacked shoots. This study was carried out on one focus pine population and our results would benefit from larger scale investigations, as well as being paralleled with a similar approach in the context of an insect epidemic. However, we are able to provide relevant support for the few studies showing that the processes at play during bark beetle endemic phases are in clear contrast to those occurring during epidemic phases (Raffa et al., 2005; Boone et al., 2011; Bleiker et al., 2014) and which generally result in overwhelmed tree-resistance mechanisms and resilience abilities (Paine et al., 1997; Kausrud et al., 2012). This work supports the potential conflicts between tree resilience abilities to endemic bark beetle attacks, and the current most highly-recommended management of bark beetle risk in forests, which involves the removal of any attacked trees to prevent the occurrence of bark beetle outbreaks (Fettig and Hilszczański, 2015).

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

	Stand		**Sub-sample**	
	Trunk 2011-2012	Shoot 2011-2012	Trunk 2011-2012	Shoot 2011-2012
Predisposing factors	DBH_{2011} $Height_{2011}$ Competition $Leaf\ Color_{2011}$ $Defoliation_{2011}$ $RW_{1950\text{-}2010}$ $WD_{950\text{-}2010}$	DBH_{2011} $Height_{2011}$ Competition $Leaf\ Color_{2011}$ $Defoliation_{2011}$ $RW_{1950\text{-}2010}$ $WD_{1950\text{-}2010}$	Ψ_{50}	Ψ_{50}
Effect variables	$Leaf\ Color_{2012}$ $Defoliation_{2012}$ $RW_{2011\text{-}2012}$ $\rho_{wood2011\text{-}2012}$	$Leaf\ Color_{2012}$ $Defoliation_{2012}$ $RW_{2011\text{-}2012}$ $\rho_{wood2011\text{-}2012}$	Ψ_{50} Weekly growth Sugars	Ψ_{50} Weekly growth

Table 2: Physiological effects (percentage and p value using a linear model) of *Tomicus destruens* attacks on trunk and shoots on Defoliation (DEF) or Abnormal Color (AC) (black) observed on *Pinus halepensis* individuals in 2011 and 2012 southeastern France. In grey, the factors that significantly not predispose to the shoot trunk attack. Ŧ indicates that the tree 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)Ŧ	32.63 (1.51e-2)Ŧ	5.53 (7.6e-2)Ŧ	16.05 (8.5e-3)Ŧ
trunk attack 2012	**ns**	40 (2.35e-3)Ŧ	**ns**	26.25 (3.41-4)Ŧ
shoot attack 2011	ns (0.75)Ŧ	ns (0.87)Ŧ	ns (0.30)Ŧ	ns (0.23)Ŧ
shoot attack 2012	**** (2.1e-2)**	21.13 (9.7e-3)	**ns**	ns (0.39)

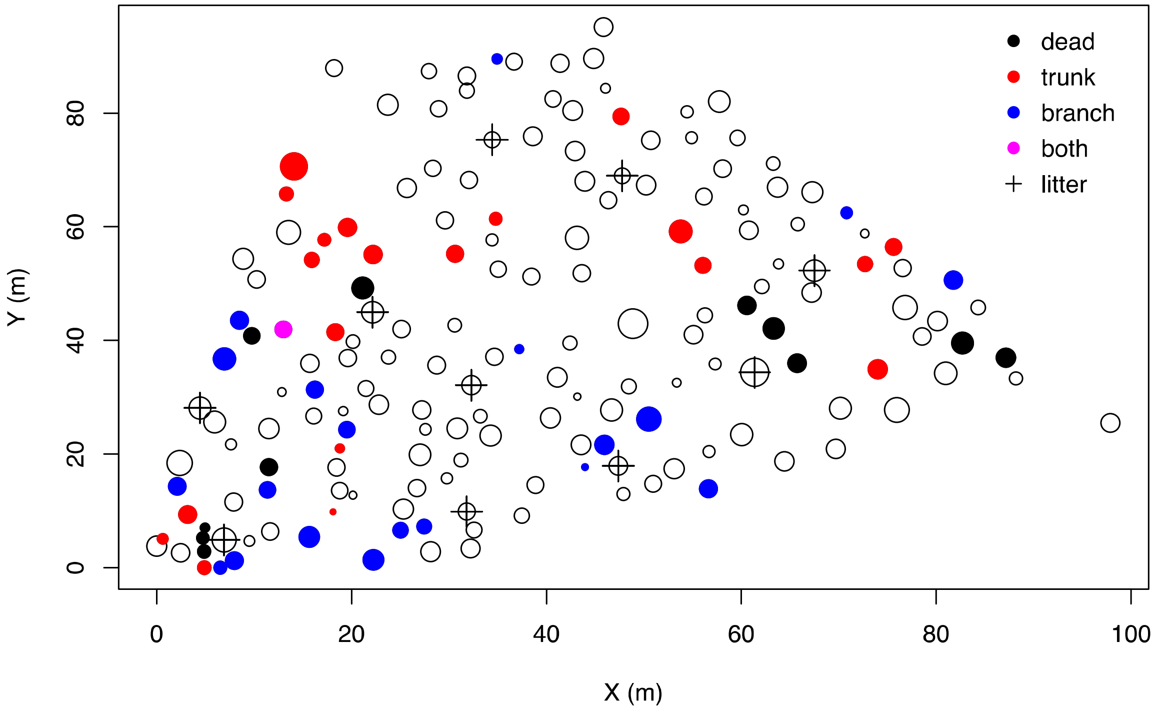

Figure 1: Map of *Pinus halepensis* individuals in the Gemenos study site, Southern France, including trees that died before 2011 (black filled circle), trunk-attacked trees by *Tomicus destruens* in 2011 or 2012 (red filled circle), shoot-attacked trees in 2011 or 2012 (blue filled circle), and one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

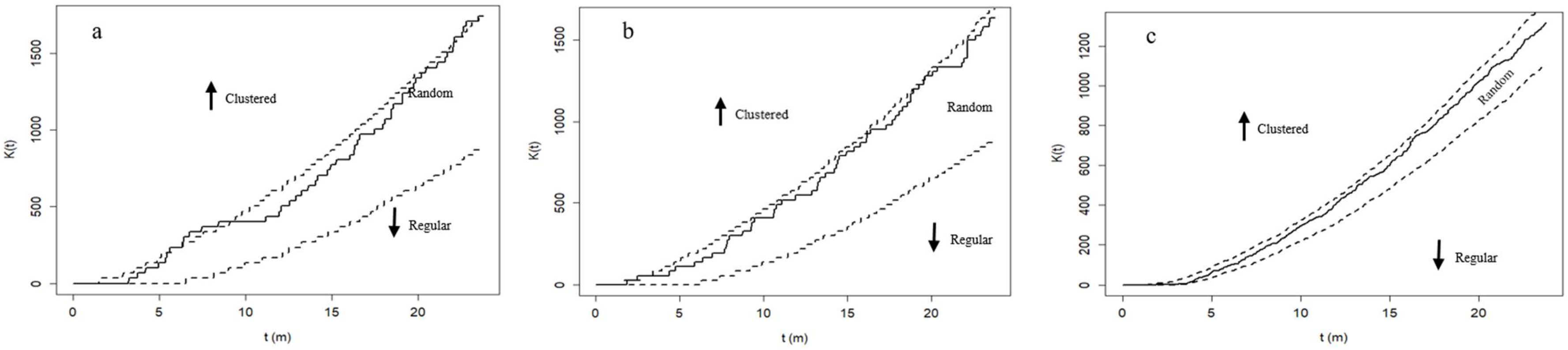

Figure 2. Univariate spatial pattern analysis using the Ripley's K-function for distributions of *Tomicus destruens* attacks in a *Pinus halepensis* population in Southern France. The figure shows the observed statistic *K(t)* for trunk attacks for the insect's breeding phase in fall 2010 (a, N=19 attacked 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 between trees *t*. Black lines give the values of *K(t)* and dotted lines give their 95% confidence envelopes. Spatial distributions of insect attacks 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, regular when *K(t)* is below the lower confidence limit.

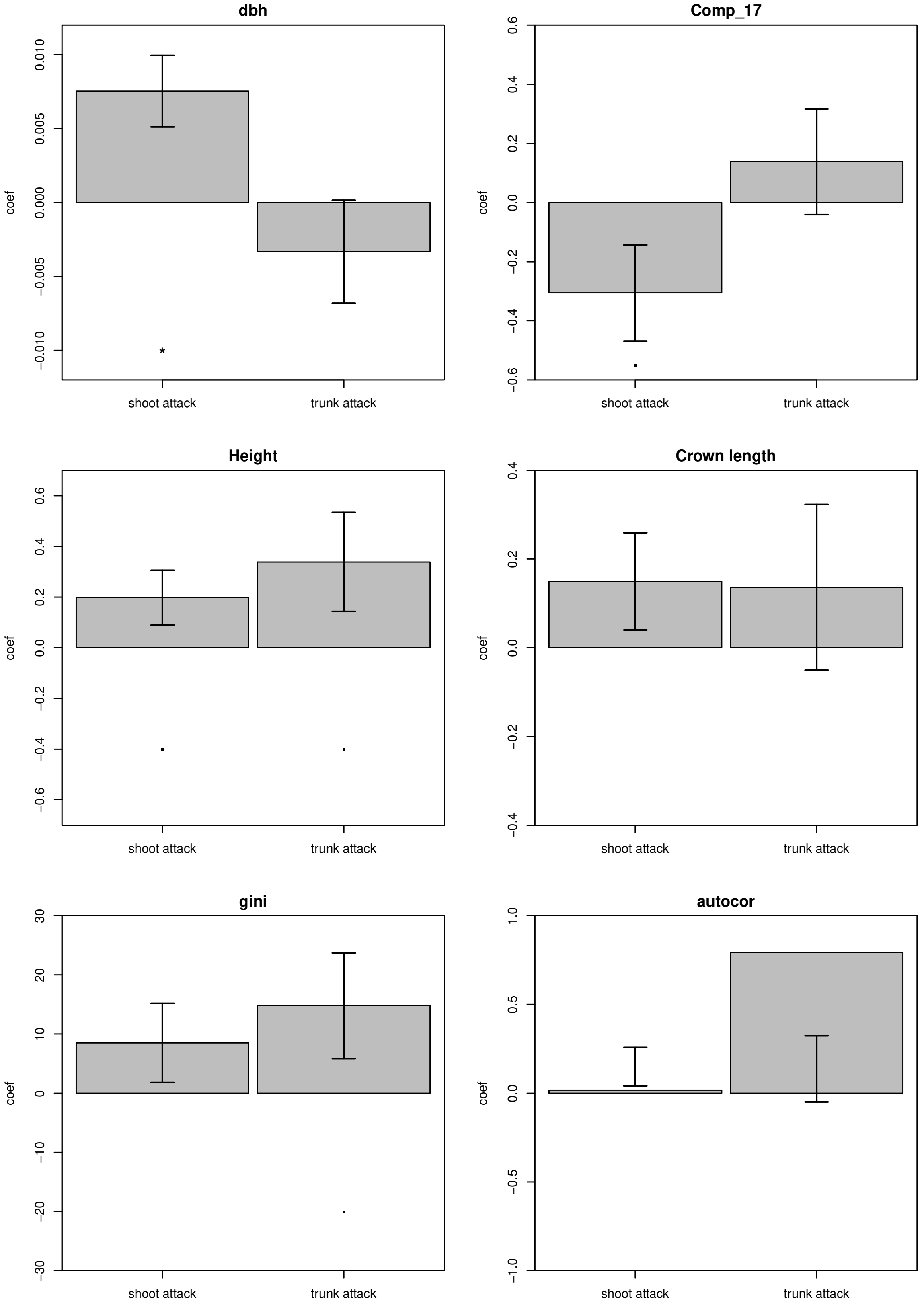

Figure 3a: Dendrometric factors predisposing *Tomicus destruens* attacks in a *Pinus halepensis* population in Southern France. Coefficient of the logistic regression analysis, non-attacked trees being taken as a reference. A star indicates that the coefficient is significantly different from the reference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

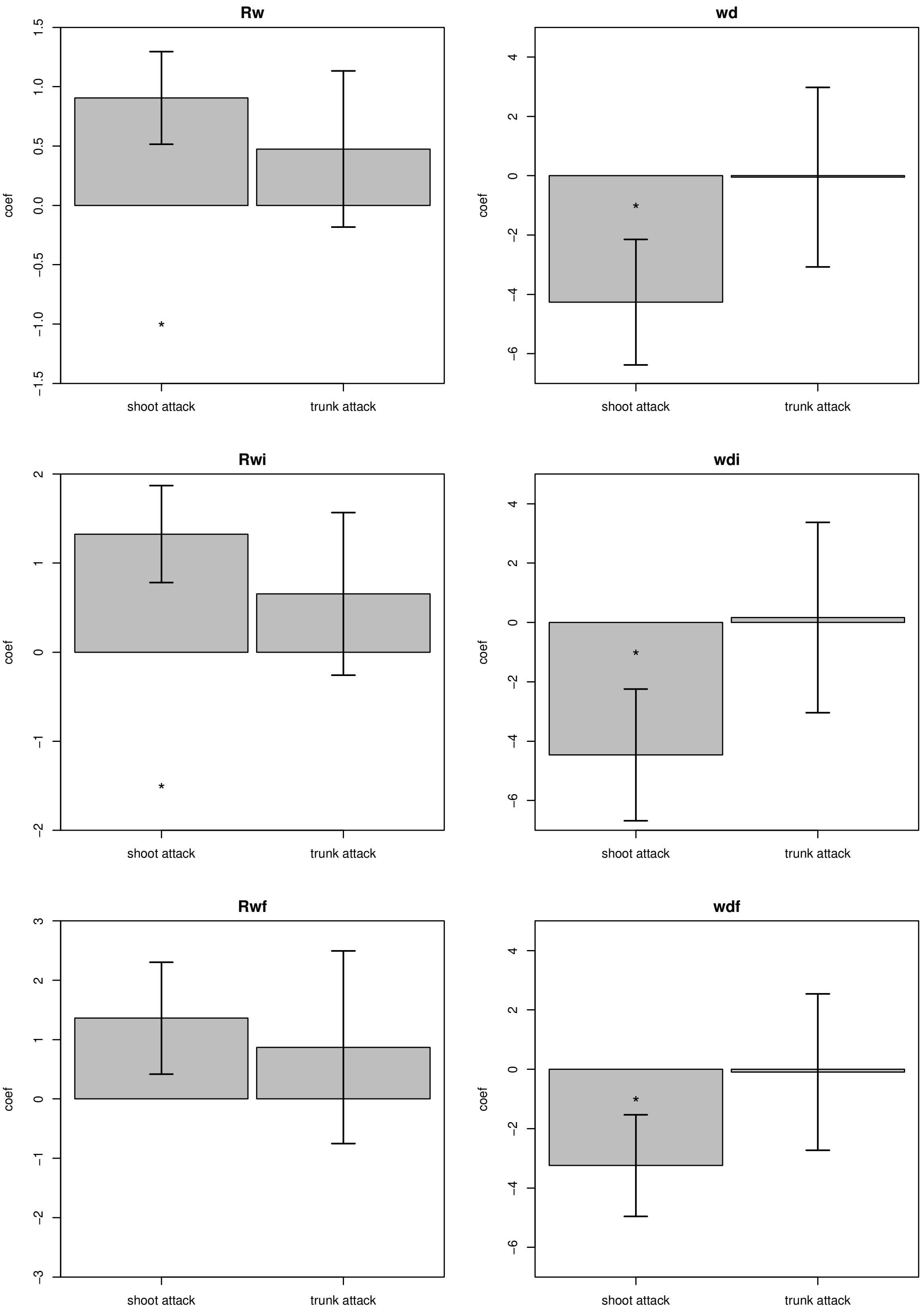

Figure 3b: Dendrometric factors predisposing *Tomicus destruens* attacks in a *Pinus halepensis* population in Southern France. Coefficient of the logistic regression analysis, non-attacked trees being taken as a reference. A star indicates that the coefficient is significantly different from the reference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

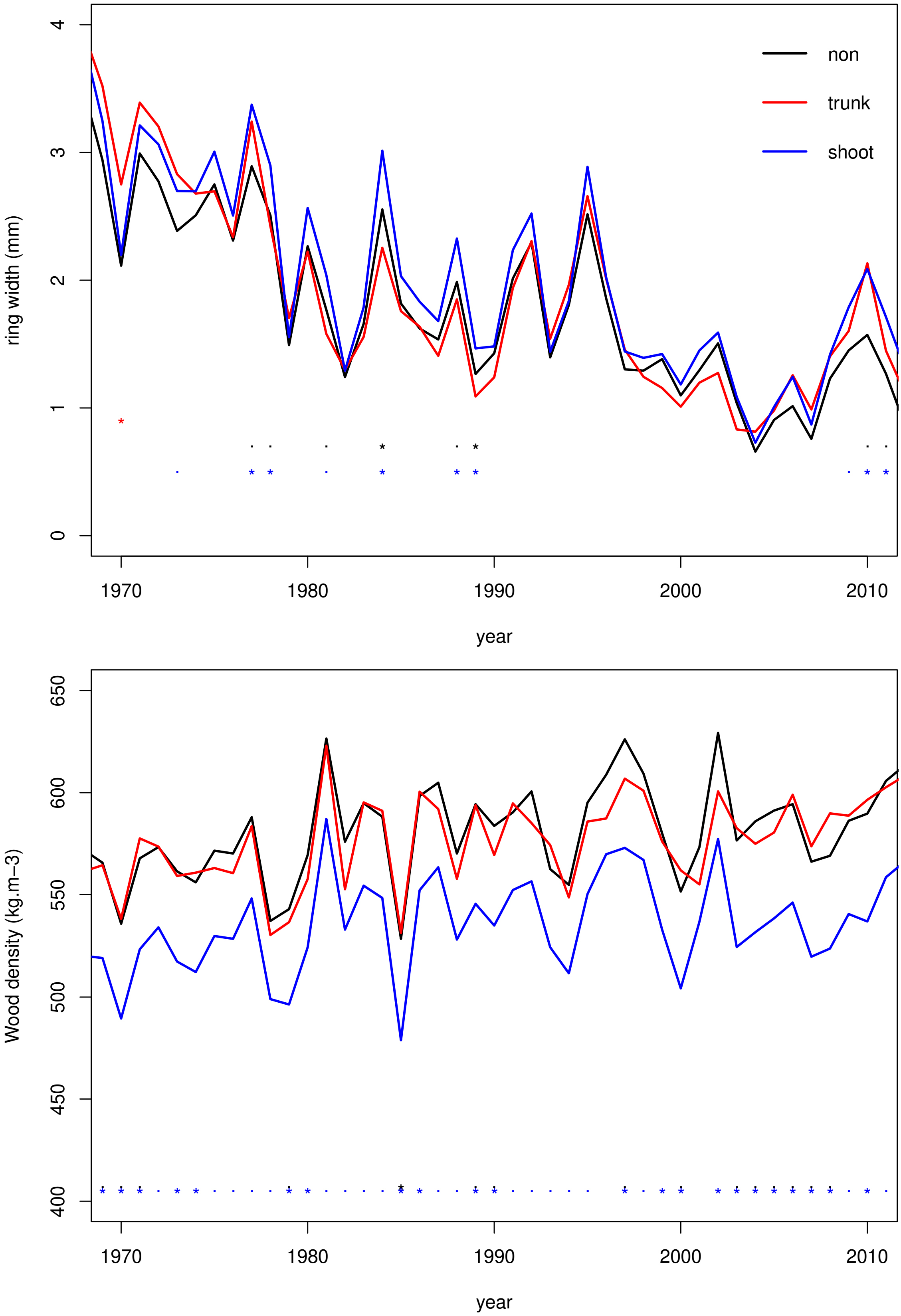

Figure 4: Past growth (ring width in mm) and wood density of non-attacked (black), shoot attacked (red) and trunk attacked (blue) individuals of *Pinus halepensis* in southeastern France. Significant differences between trunk attacked trees and non-attacked trees in red (* at 5% and at 10%). Significant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

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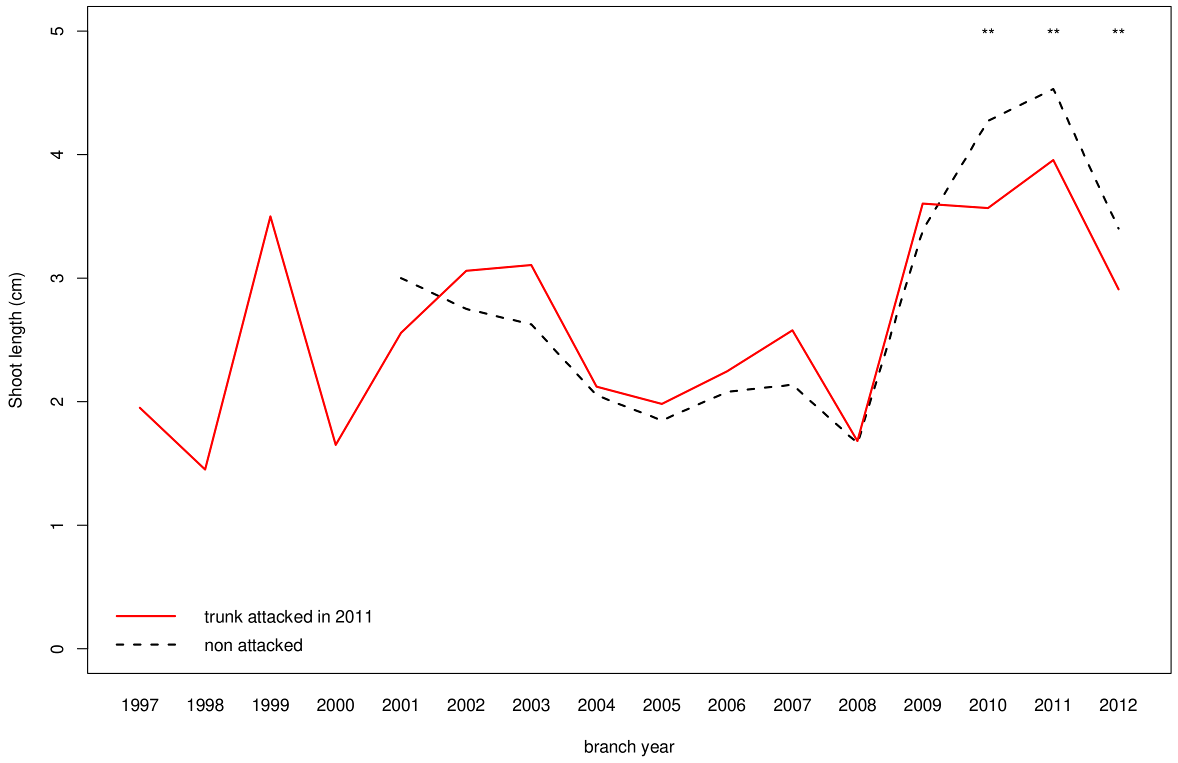

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788 **Figure 5:** Shoot length (cm) of *Pinus halepensis* individuals that were attacked and non-attacked by *Tomicus destruens* in 2011 in southeastern France.

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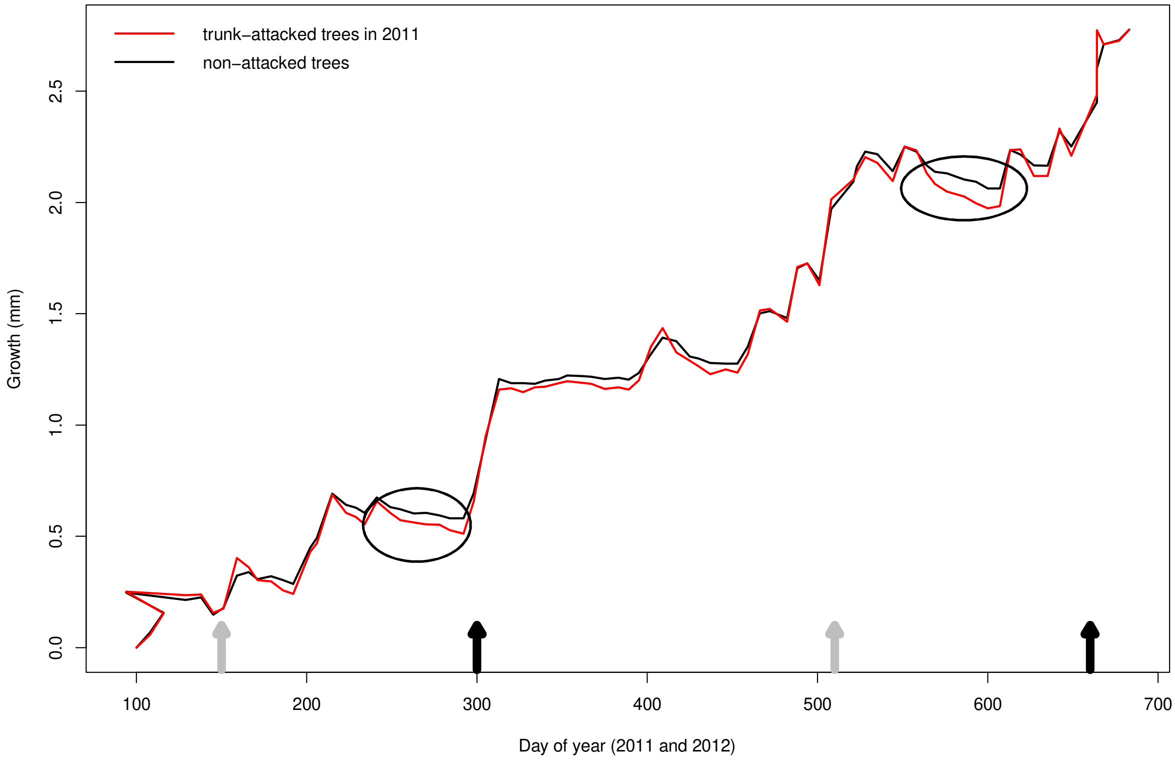


Figure 6: Effect of *Tomicus destruens* trunk attacks on *Pinus halepensis* weekly growth measured using hand-held micro-dendrometers in 2011 in southeastern France. The black ellipses represent the period of shrinkage of the trunk. The black arrow represents the trunks attacks and the grey arrows the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

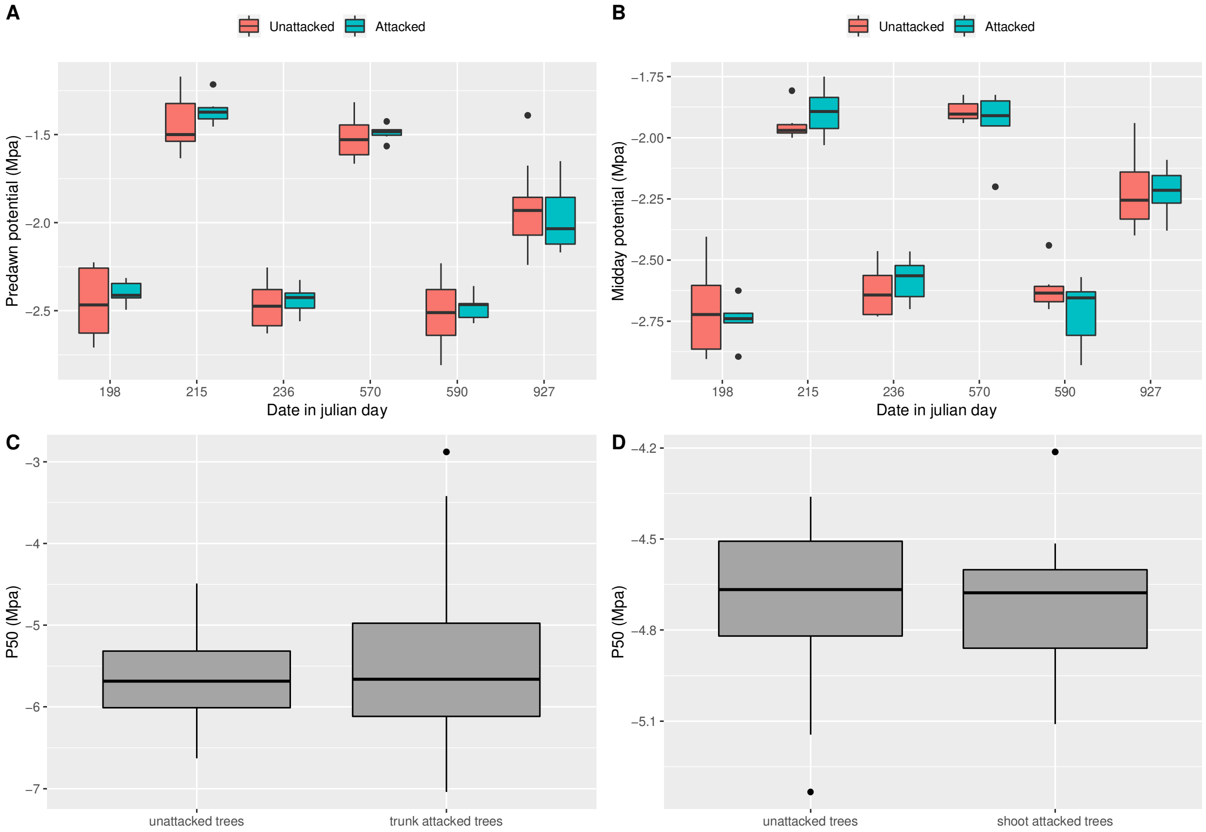


Figure 7: (a, b) Effect of 2011 trunk attacks on predawn and midday water potentials dynamics (Mpa) on *Pinus Halepensis* trees. **(c, d)** Effect of trunk attacks (in 2011) and shoot attacks (in 2012) by *Tomicus destruens* on *Pinus Halepensis* xylem water potential inducing 50% of conductivity (P50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

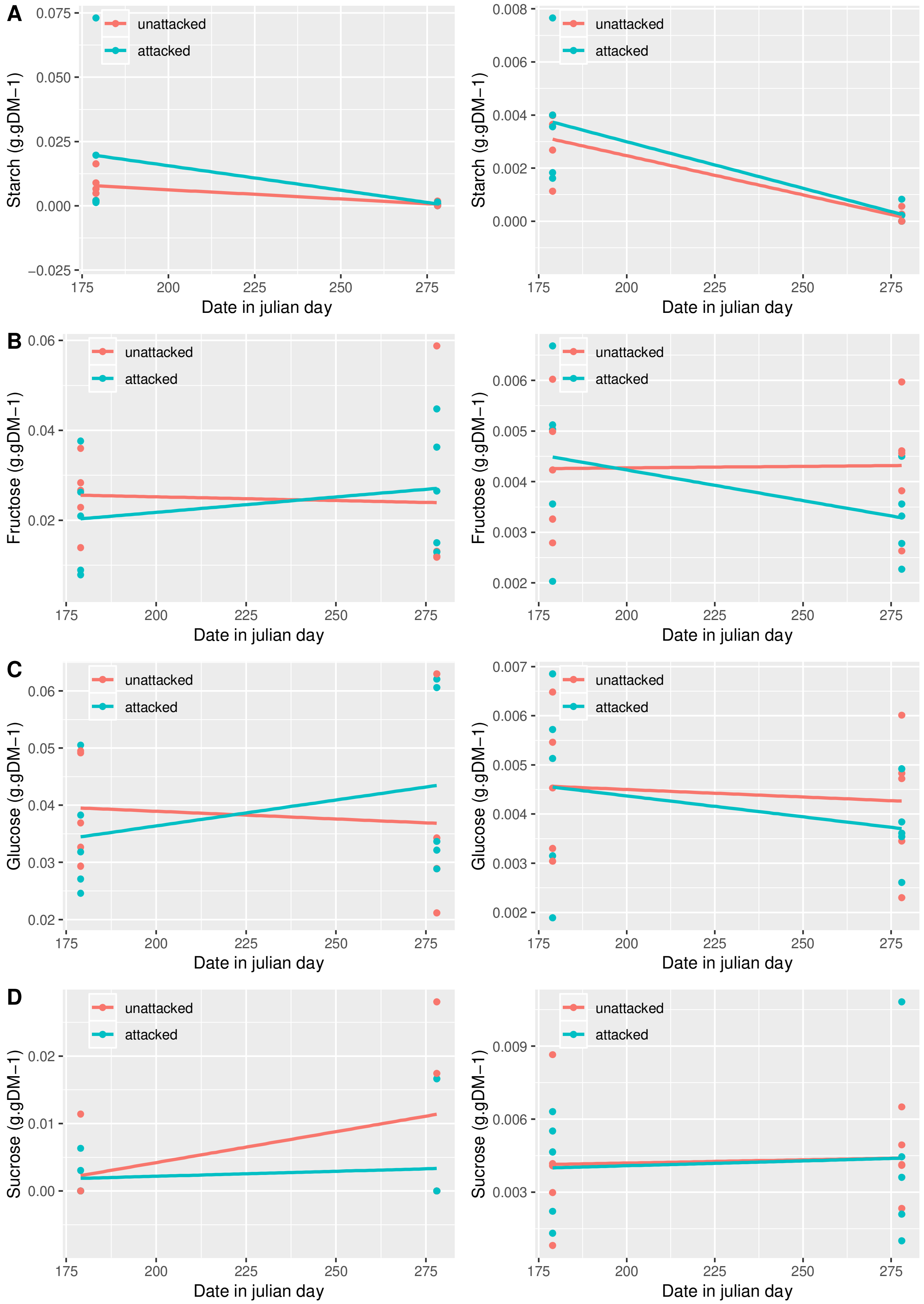


Figure 8: Non structural carbohydrates concentration (g g_{DM}^{-1}, **starch, fructose, glucose, sucrose) in *Pinus halepensis* trunk phloem** (left) **and xylem** (right) before and after trunk attacks in 2011 for trees attacked by *Tomicus destruens* (red long dotted line) and non-attacked (black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

Supplement Figures

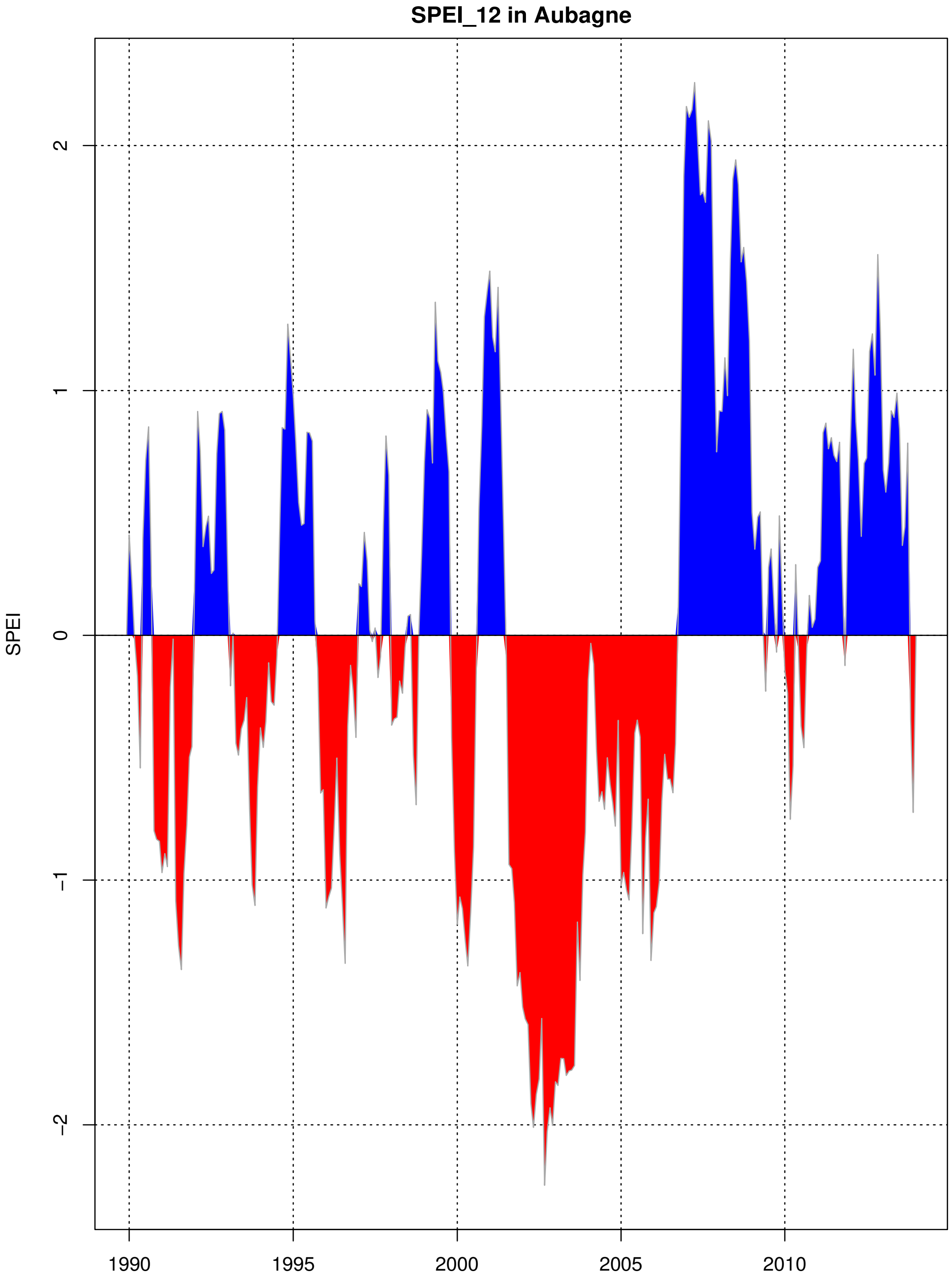


Figure s1: Dynamics of 12-months Standardized Precipitation-Evapotranspiration Index measured in Aubagne, a locality located 5 km from the study site. In red the drought periods and in blue the wet periods.

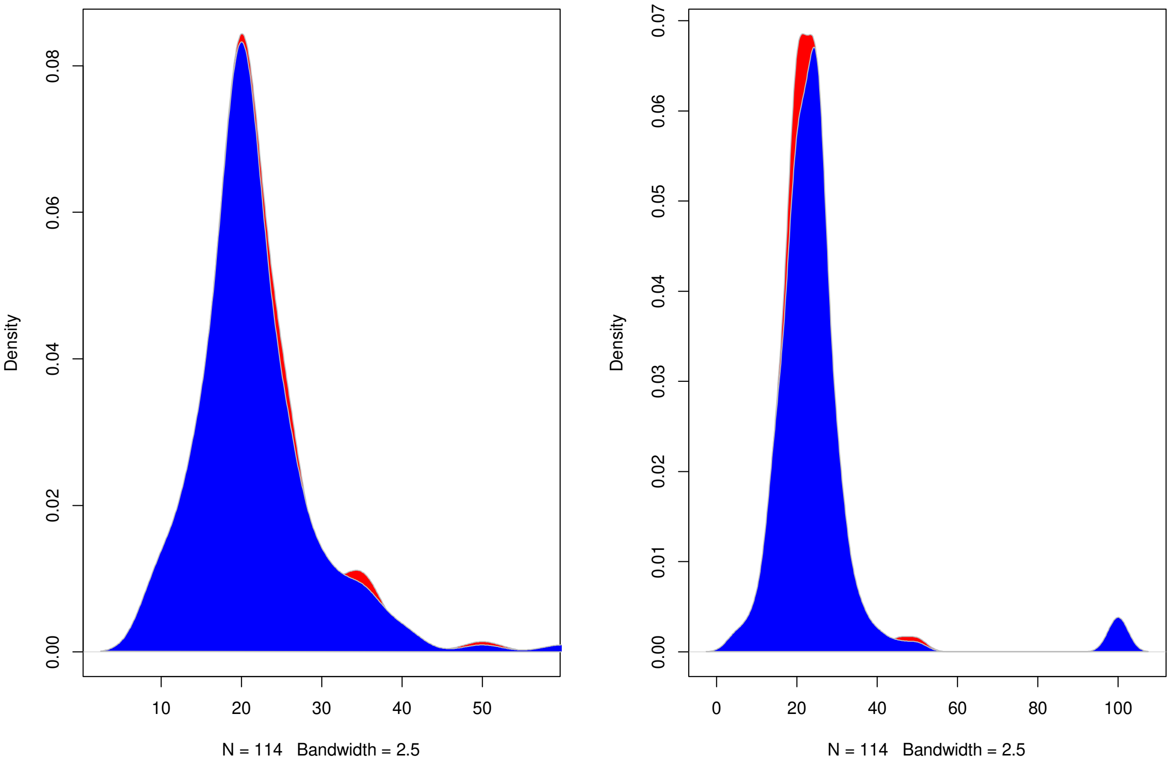


Figure s2: Comparison of defoliation class distributions between the subsample of the all monitored *Pinus halepensis* trees (blue) and the subsample of *Pinus halepensis* trees, whose cores were analyzed (red).

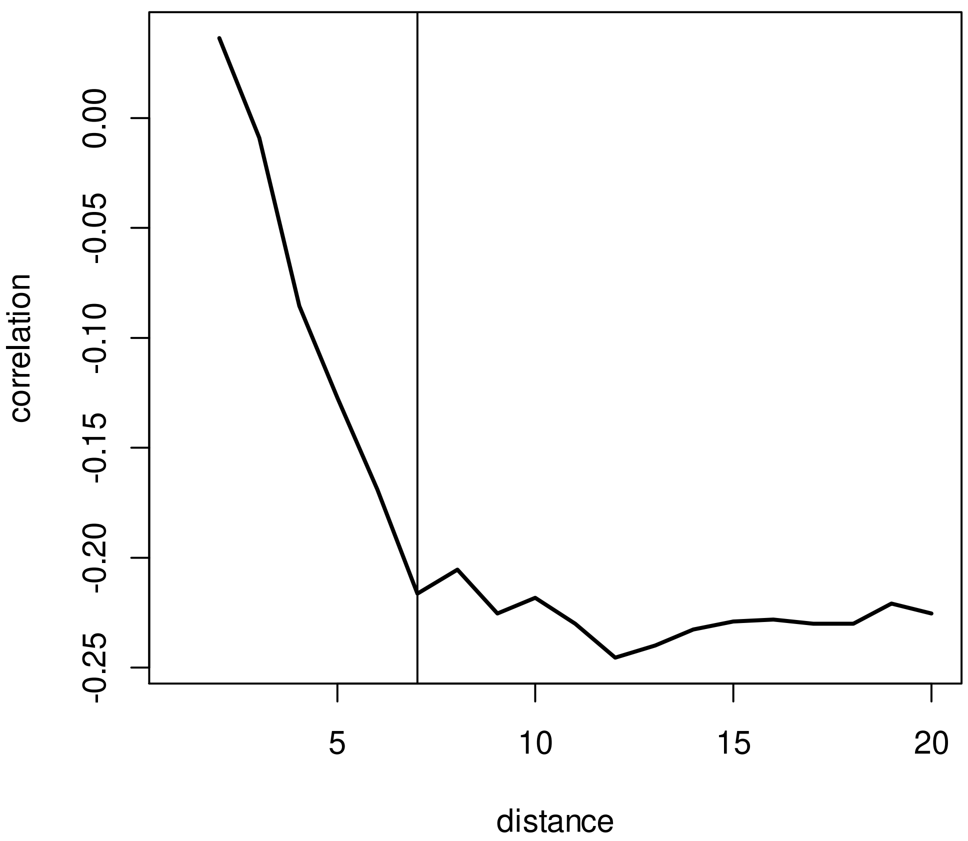
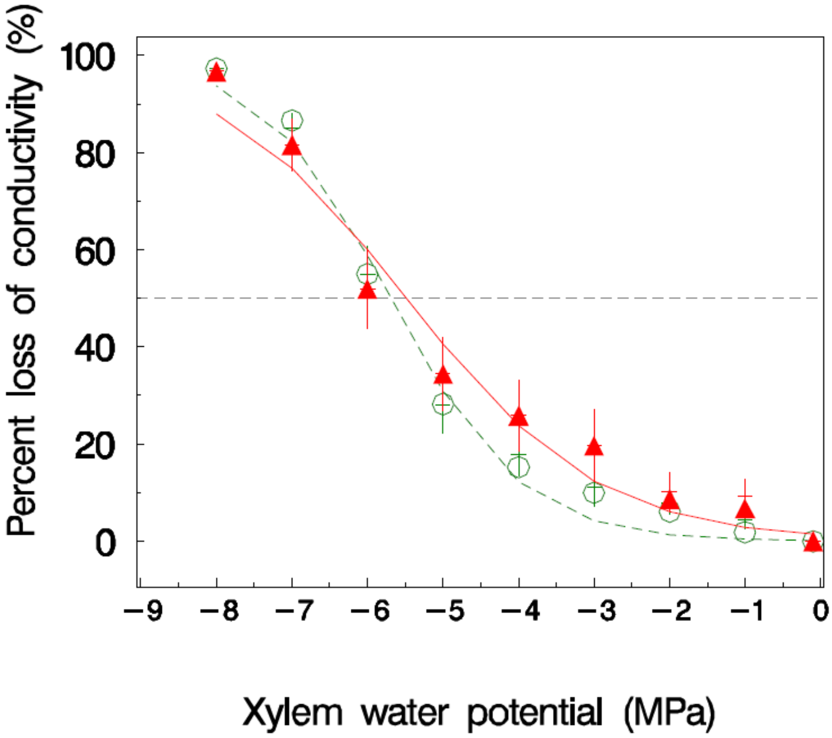


Figure S3: correlation coefficients (r) between the Martin-Ek competition index at a given distance of competitive trees and growth increment in a southeastern French *pinus halepensis* population between 2011 and 2012.



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.