Forest Ecology and Management 274 (2012) 201–209

Contents lists available at SciVerse ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Understanding the role of sapwood loss and reaction zone formation on radial growth of Norway spruce (*Picea abies*) trees decayed by *Heterobasidion annosum* s.l.

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ARTICLE INFO

Article history:
Received 20 October 2011
Received in revised form 24 January 2012
Accepted 16 February 2012

Keywords:
Sapwood
Heartwood
Reaction zone
Resistance
Tolerance
Dendrochronology

ABSTRACT

Allocation of photosynthates to defence responses at the expense of biomass increase is a common strategy amongst plants to cope with stress factors. Trees reduce the spread of decay by creating a secondary metabolite-rich reaction zone as fungal ingresses the sapwood. Reaction zone formation implies a sacrificial conversion of sapwood, thus, as decay progresses, the sapwood area of the tree is reduced. The relative contribution that reaction-zone formation and sapwood loss make to radial growth decrease is unclear. To answer this question we reconstructed radial-growth patterns in 100 Norway spruce (*Picea abies*) trees with a range of reaction zone and sapwood disruption. Basal area increment (BAI) between 1960 and 2007 and its relationship with sapwood reduction and reaction zone formation was assessed using structural equation models (SEM). BAI data showed that over 10 years, trees with small or no decay columns (<40%) and a reaction zone shifted from a growth rate that was similar to trees without a reaction zone towards low growth rate similar to trees with large decay columns. The fitted SEM indicated that: (i) the effects of decay on growth would begin with the formation of the reaction zone, and (ii) the smaller sapwood in decayed trees as compared with healthy trees would not reduce radial growth, but would be in part the result of previous periods of low growth due to reaction-zone formation.

1. Introduction

Plants divert resources from biomass production into defence, which increases their chance of overcoming herbivore and pathogen attacks; however, it also decreases their ability to grow and outcompete other plants (Herms and Mattson, 1992). In trees, the trade-off between growth and defence can be inferred from positive correlations between lignin biosynthesis, starch accumulation and reduced growth (Novaes et al., 2010). Root and butt rot pathogens are amongst the most destructive organisms in forest ecosystems, playing a major role in their dynamics (Garbelotto, 2004). Trees have several defence mechanisms against wood decay fungi that may or may not imply additional energy costs (Pearce, 1996). Wood structure has several attributes that constitutively prevent spread of decay in axial, radial and tangential directions (Shigo and Marx, 1977). Decay progression can be regarded as similar to air entering the conductive cells of the sapwood, resulting in the physical closure of pit apertures, without energy consumption (Zimmermann, 1983). However, most trees can create reaction zones, in which the wood is physically and chemically modified partly through the activation of living parenchyma cells (Shigo and Marx, 1977; Schwarze et al., 2000). The formation of a reaction zone implies *de novo* synthesis of secondary metabolites with obvious energetic costs. The advance of decay may not be completely stopped by a reaction zone because not all fungi are equally susceptible to its fungistatic substances and decay can spread during tree dormancy (Schwarze and Baum, 2000; Baum and Schwarze, 2002).

The energetic costs of reaction-zone creation can have economic consequences and, therefore, might be of interest when breeding for decay resistance in economically important species such as Norway spruce [*Picea abies* (L.) H. Karst]. Norway spruce is highly susceptible to the root and butt rot fungus *Heterobasidion annosum* s.l. (Fr.) Bref. The fungus enters the tree via root-to-root contacts with neighbouring infected trees or stumps, and spreads through the heartwood forming decay columns of up to 10 m (Stenlid and Redfern, 1998). Spruce are light-coloured heartwood trees or ripewood trees, in which heartwood has less moisture than sapwood, without posing a constitutive barrier to fungal invasion (Schwarze et al., 2000). The induction of a strong defence response when attacked by pathogens may compensate for the low level of constitutive defences. This process is exemplified by the Norway spruce response to infection by *H. annosum*: the infection is compartmentalized by the creation of both physical and chemical barriers. In between the mycelial front and the sapwood, infected trees have a dry zone where rays and tracheids show embolism

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http://dx.doi.org/10.1016/j.foreco.2012.02.026

and appear enriched with phenolic compounds (Johansson and Stenlid, 1985). This transition zone is subsequently infiltrated with fungistatic compounds (Stenlid and Johansson, 1987) until approximately a year later when it turns into a reaction zone (Shain, 1971). The high pH and the high concentration of several lignans such as hydroxymatairesinol (up to 6% of dry weight) in the reaction zone partly prevent the growth of the pathogen (Shain and Hillis, 1971).

Growth losses due to decay in Norway spruce are well documented. Bendz-Hellgren and Stenlid (1995) compared 329 pairs of trees from the National Forest Inventory of Sweden and observed that decayed trees grew less than healthy ones. They hypothesized that 50% of the growth loss could be due to the formation of a reaction zone. In a later study, in which 1610 trees were inspected, it was shown that trees with decay and a reaction zone grew less than the healthy trees; however, decayed trees without a reaction zone showed growth rates that were similar to that of the healthy trees (Oliva et al., 2010). Other processes that occur alongside reaction-zone formation could also explain growth reductions given that decay advance may impair water transportation efficacy, disturb translocation of photosynthates towards the root system and reduce the water storage capacity of the stem. Growth losses have been also associated with severe root damage and thinner crowns (Froelich et al., 1977; Kelsey et al., 1998). In Norway spruce, thinner crowns have been shown to correlate well with higher decay columns (Vollbrecht and Agestam, 1995), which are typically associated with a lower growth rate (Solberg, 1999). Nevertheless, the relative effect of the disruption of the conductive system on the formation of the reaction zone has not been evaluated yet. Sapwood size corresponds well with the size of the living crown (Longuetaud et al., 2006), thus it may be used as a quantitative measure of root and crown disruption (Gould and Harrington, 2008).

The aim of the present study was to understand the mechanisms behind the decrease in radial growth due to decay development in Norway spruce. First, we tested the contribution of reaction-zone formation to radial-growth decrease by comparing basal area increment (BAI) between decayed trees with different degrees of reaction zone and decay size. Second, we used structural equation models (SEM) to test the following hypotheses: (i) trees reduce their radial growth because they allocate resources into the reaction zone, (ii) trees create a reaction zone thus reducing their sapwood cross-sectional area, which reduces their growth rate, and (iii) trees create a reaction zone hence decreasing their growth rate, which results in a reduced production of sapwood.

2. Material and methods

2.1. Sampling sites and section measurements

Three managed stands of Norway spruce (*Picea abies*) located in Central Sweden were studied: Ingbo, Enåker and Harsbo (Table 1). In 2009, 500 trees were felled and screened for the presence of decay at stump level (height ca. 0.30 m). Those trees showing signs of decay at stump height were further inspected at 0.66 m and at breast height (1.30 m). The proportion of each section corresponding to sapwood, heartwood and reaction zone was calculated based on pixel counts after manual classification in Adobe Photoshop CS3 Extended (version 10.0.1) (Fig. 1). According to our classification, sapwood corresponded only to healthy conductive functional sapwood, and heartwood to un-decayed sound heartwood. We could not assign decay as belonging to either heartwood or sapwood, therefore decay included all tissues with wood discoloration regardless the position in the cross-section. Sections were sprayed with a pH indicator: 2,6 dichlorophenolindophenol. Areas with a high pH that did not correspond with the reaction zone that had been identified by naked eye were also measured and considered to be an 'incipient reaction zone'. A slice of each tree was stored in the dark in a plastic bag and after two weeks the presence of *Heterobasidion annosum* s.l. conidiophores was noted.

2.2. Dendrochronology analyses

All decayed trees and a random subsample of 17 healthy trees were considered for dendrochronological measurements. Trees with few visible rings were discarded, likewise trees with old decay pockets (hollow trees) affecting the first rings. Trees in which decay was only observed at breast height and not at stump level, indicating rot coming from wound infection instead of root rot, were also discarded. In total, 100 trees were measured with varying amounts of decay at stump level (% of the section): 17 healthy trees with 0% decay, 42 trees with >0–10% decay, 22 trees with >10–40% decay and 19 trees with >40–80% decay. Growth measurements were performed using images of cleanly-cut stem sections taken at breast height. Tree-ring width was measured along two radii to an accuracy of 0.01 mm using the semi-automated software CooRecorder v. 7.4 (Larsson, 2011). Preliminary matching of the two radii measured in each stem was visually performed using CDendro v. 7.4 (Larsson, 2011). The quality of the visual cross-dating was checked using the program COFECHA (Holmes, 1983). We corrected the trend of decreasing tree-ring width with increasing stem size by transforming ring-width data into BAI. In trees in which the early rings could not be measured because of decay, the distance between the innermost tree ring and the pith was added into the calculations of BAI. In trees in which the pith was lost, we reconstructed the approximate location of the pith by geometric methods assuming a constant growth rate of the inner rings (Rozas, 2003).

2.3. Sapwood eccentricity

Reaction zone formation often results in a narrowing of the sapwood in the vicinity of the decay column. On the other side of the stem, a compensatory widening of the sapwood occurs. The bias of the sapwood centre from the stem centre was defined as sapwood eccentricity. From the same trees used in the dendrochronological analyses, 153 sections corresponding to 70 trees were used to calculate the eccentricity of the sapwood versus the non-conductive part of the section (NCS). The NCS consisted of a reaction zone, an incipient reaction zone, heartwood and decay. Only sections

Table 1
Characteristics of the studied stands and number of samples.

Site	Geographical coordinates		Age (years)	Average diameter (cm) (min–max)	Decay incidence (% of trees)	*H. annosum* incidence (% of decayed trees)	Number of trees (sections)	
	Latitude (N)	Longitude (E)					Section image classification	No. trees measured
Ingbo	60° 07′	16° 47′	83	23.9 (12.0–40.0)	26.8	73.7	83 (236)	65
Enåker	60° 04′	16° 46′	88	17.4 (9.0–38.0)	20.2	72.2	39 (91)	20
Harsbo	60° 23′	17° 22′	72	18.0 (8.0–31.0)	14.0	38.5	35 (72)	15

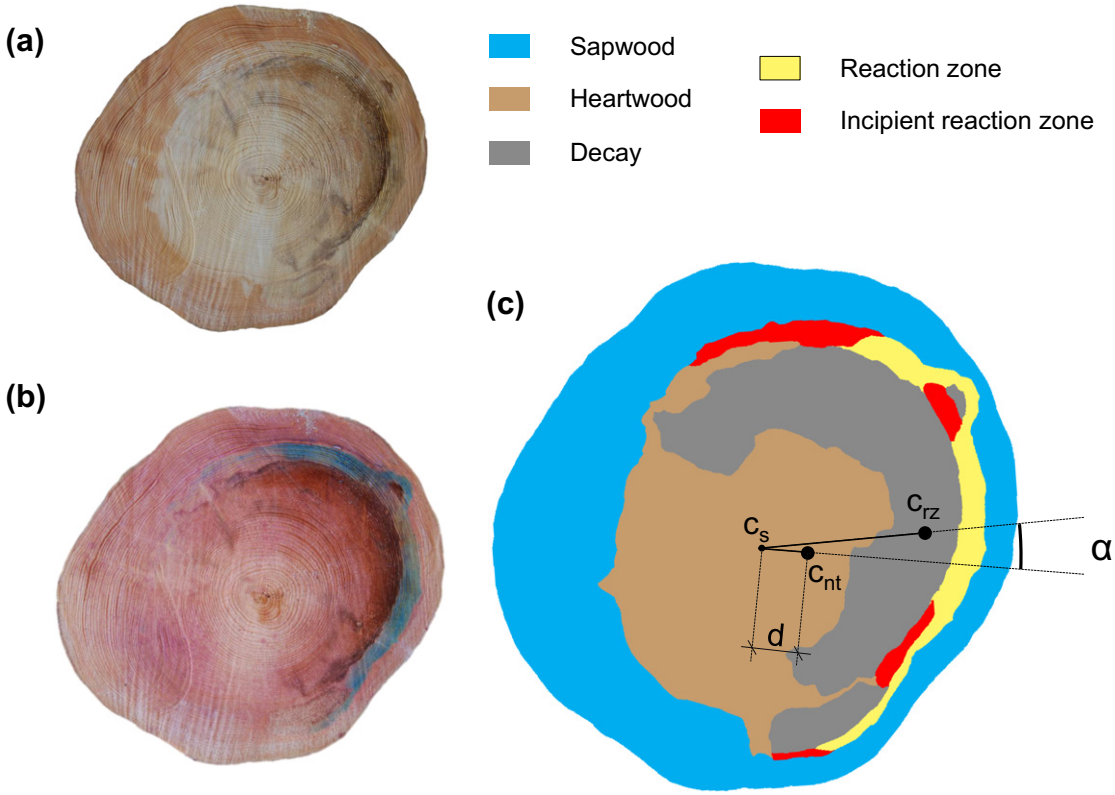

Fig. 1. Example of image classification and sapwood eccentricity measurements. (a) Freshly cut slice and, (b) slice sprayed with 2,6-dichlorophenolindophenol. (c) The shift of sapwood due to the lateral decay column 'd' is calculated as the translation of the centroid of the section 'c_s' and the centroid of the non-sap conductive part of the section 'c_{nt}' corresponding to the area occupied by the decay, heartwood, reaction zone and incipient reaction zone. The angle 'α' between the vector from 'c_s' and the centroid of the reaction zone 'c_{rz}', and the vector from 'c_s' to 'c_{nt}' is used as a measure of the alignment between the reaction zone and the sapwood shift. A high degree of alignment indicates an association between reaction zone and sapwood alteration.

with decay and a reaction zone were included. We used the angle between the sapwood and the reaction zone to relate sapwood eccentricity with reaction zone formation. We constructed two vectors, one between the centroid of the NCS and the centre of the section, and a second between the centroid of the reaction zone and the centre of the section (Fig. 1). Smaller angles would imply alignment and large angles would imply no relation. Calculations of the origin from the centroid of each area were performed with the image analysis software Assess (American Phytopathological Society, St. Paul, MN, USA). In the case of sections with multiple reaction zone parts, only the largest part was selected. Centroids are automatically computed with Assess based on the boundary of the shape, which allows calculation of centroids even in concave shapes. Since calculations are based on the contour of the object, sections whose reaction zone had entirely circled the NCS were excluded, since both the centroid of the NCS, and the centroid of the reaction zone, coincided.

2.4. Statistical analyses

According to the association between sapwood and decay, trees were sorted into trees with large decay columns (>40% of the section) and trees with small decay columns (<40% of the section). Differences in relative proportion of the cross-section area belonging to sapwood, heartwood and reaction zone between these types of trees and regression coefficients were considered significant at $p < 0.05$. We tested differences in growth between different classes of trees at Ingbo with a repeated-measures analysis (Schabenberger and Pierce, 2002). An autoregressive structure for the variance–covariance matrix gave the best fit. The mean BAI chronologies were used to explore growth, diameter and sapwood differences between trees with large decay columns and small decay columns with and without reaction zone. Within the age range of the trees of this study (Table 1), sapwood width is considered to be constant with the age of the tree (Sellin, 1994) and, hence, sapwood differences were tested in terms of average sapwood width (cm). The analyses were carried out using the MIXED procedure of SAS/STAT software v. 9.1.

2.5. Structural equation modelling

We used SEM to test different mechanistic hypotheses explaining growth losses due to decay. Four variables were used: (i) proportion of decay in the section, (ii) proportion of reaction zone (following *logit* transformation), (iii) basal area in 1980 (used as a surrogate of tree dominance), (iv) average growth (BAI) from 1997 to 2007, and (v) mean sapwood width. In the first analysis, we tested previous findings (Oliva et al., 2010) by comparing the association between decay and growth alone, with the inclusion of an indirect effect of the formation of a reaction zone on growth. In the second analysis, we fitted and compared two models: (i) the 'sapwood reduction' model in which growth was affected by the effect of decay eliciting a reaction zone replacing the sapwood, and (ii) the 'defence allocation' model in which the tree produces a reaction zone thus decreasing its radial growth, which results in a smaller sapwood. In both models, we allowed the reaction zone to affect the sapwood directly to include the effect of the reaction zone physically substituting the sapwood (i.e. the tree creates a reaction zone on the same sapwood tissue). Given that sapwood width can vary between dominant and suppressed trees (Sellin,

1994), as well as with the variables mentioned above, all models included a path between basal area in 1980 and sapwood width. The models were first fitted in all locations, and tested independently in each location for validation. We used the Chi-square statistic (χ^2), the root mean square error (RMSEA) and the Akaike information criterion (AIC) to assess the models' goodness-of-fit. When fitting SEMs, high p values are preferred over low p values because high p values indicate the likelihood that differences between the theoretical model and the observed covariance values are due to chance alone. We present the results as standardized path coefficients. In a path X → Y, the coefficient (a_{xy}) represents the standardized effect of increasing or decreasing X into Y (Grace, 2008). The increase is measured in terms of standard deviation (i.e. an increase of one standard deviation in X will produce an a_{xy} increase in terms of standard deviation in Y). The significance of direct and indirect effects was tested by means of Bayesian analyses. Structural equations were adjusted with AMOS v. 19.0.0 (Amos Development Corporation, Meadville, PA, USA).

3. Results

3.1. Sapwood, heartwood and decay

Large decay columns were associated with a reduction of the proportion of conductive sapwood (Fig. 2). The loss of sapwood as decay increased was similar at the three levels assessed (0.30 m, 0.66 m and 1.30 m) (Fig. 2a) and comprised two phases: up to 40% decay and more than 40% decay (Fig. 2b). When observing trees at early stages of decay development, decay increases correlated poorly with a decrease in sapwood, but when decay comprised about 40% of the section (Fig. 3), further increases in the proportion of decay correlated with a strong decrease in the proportion of sapwood (Fig. 3). When decay reached the 40% of the section, decay had spread into most of the heartwood. Accordingly, when the decay covered more than 40% of the section, no or very little sound heartwood was left in the section on average (2.2% of sound heartwood in severely decayed trees (>40% decay) versus 33.0% of sound heartwood in trees with <40% decay, $p < 0.0001$).

When observing trees with differing proportions of decayed wood, it appeared that, as decay columns become larger, trees tended to show a reaction zone in-between the sapwood and the decay. The size of the reaction zone correlated with the size of the decayed area in trees where the latter covered approximately 40% of the section ($R^2 = 0.36$, $p < 0.0001$). In trees beyond this level of decay (>40% of the section), the reaction zone area remained constant ($R^2 = 0.01$, $p = 0.50$) (Fig. 2).

In parallel with the formation of a reaction zone, a local reduction in the proportion of sapwood (Fig. 3) resulting in the eccentricity of the sapwood area was observed (Fig. 4). In trees at early stages of decay (<20% of the heartwood), this process did not associate with major changes in the architecture of the sapwood. But, in trees with increasingly larger decay columns (20–60%), the formation of the reaction zone was associated with sapwood eccentricity as sapwood became narrower close to the reaction zone. Up to a 40% of decay, a larger size of the reaction-zone did not translate into a proportional decrease of sapwood (Fig. 3), instead trees with a reaction zone tended to have consistently smaller sapwoods (linear and exponential function, $R^2 = 0.13$ and 0.31, respectively). In trees with decay columns comprising less than 40% of the section, tree sections with a reaction zone had a smaller proportion of sapwood than sections without a reaction zone (49.1% versus 60.8%, $p < 0.0001$). In trees with decay covering more than 40% of the section, no more sapwood eccentricity was observed (Fig. 4), and increases in the area of decay correlated with reductions in the proportion of sapwood (Fig. 3).

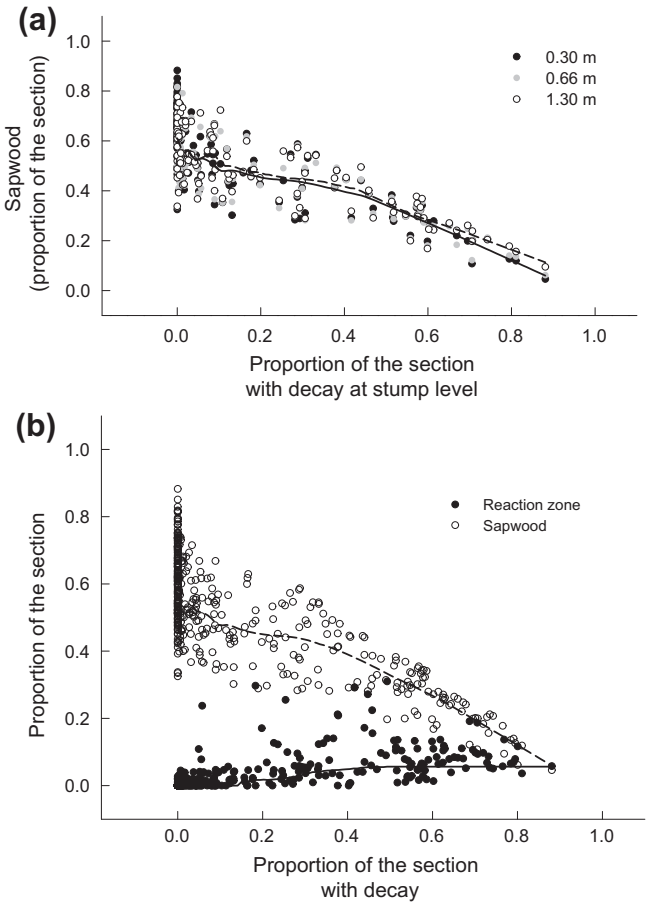

Fig. 2. Association between decay and sapwood size in spruce trees. (a) Association between sapwood size at 0.30 m, 0.66 m and 1.30 m and decay at stump level (0.30 m). Lines represent data smoothed following LOESS smoothing at 0.30 m (solid line) and at 1.30 m (dashed line). (b) Association between decay and the proportion of the section with a reaction zone or sapwood. Lines represent LOESS smoothing for sapwood (dashed line) and moving median for the reaction zone (solid line). A sampling window of 20% of the samples was used for both LOESS and median smoothing.

3.2. Growth and decay

At Ingbo, the tree-ring profile of 65 trees revealed a low and steady rate of growth until 1969, which was followed by an increase in growth rate following the first thinnings that peaked in 1980. This was followed by a period of slower growth until 1991, followed by another increase in growth rate until 2007 (Fig. 5). Between 1980 and 2007, trees with a reaction zone grew less than trees without a reaction zone ($p = 0.015$). Amongst decayed trees, trees with small decay columns (<40%) grew more than trees with large decay columns (>40%) ($p = 0.0056$). Amongst trees with small decay columns, trees with larger reaction zones tended to grow less ($p = 0.0033$), but no relationship was found between decay size and reaction zone and the amount of tree growth ($p = 0.13$). Amongst trees with large decay columns, no correlation between growth and reaction zone size was found ($p = 0.29$). Trees where the entire heartwood had decayed grew less than trees that had some un-colonized heartwood ($p = 0.038$).

Up until 1980, trees with a reaction zone showed the same growth rate as trees without a reaction zone. However, between 1980 and 2007, trees in the early stages of decay that had formed a reaction zone showed an intermediate growth rate between trees without a reaction zone and trees with a large decay column ($p = 0.0095$). By the time that their last growth rings were mea-

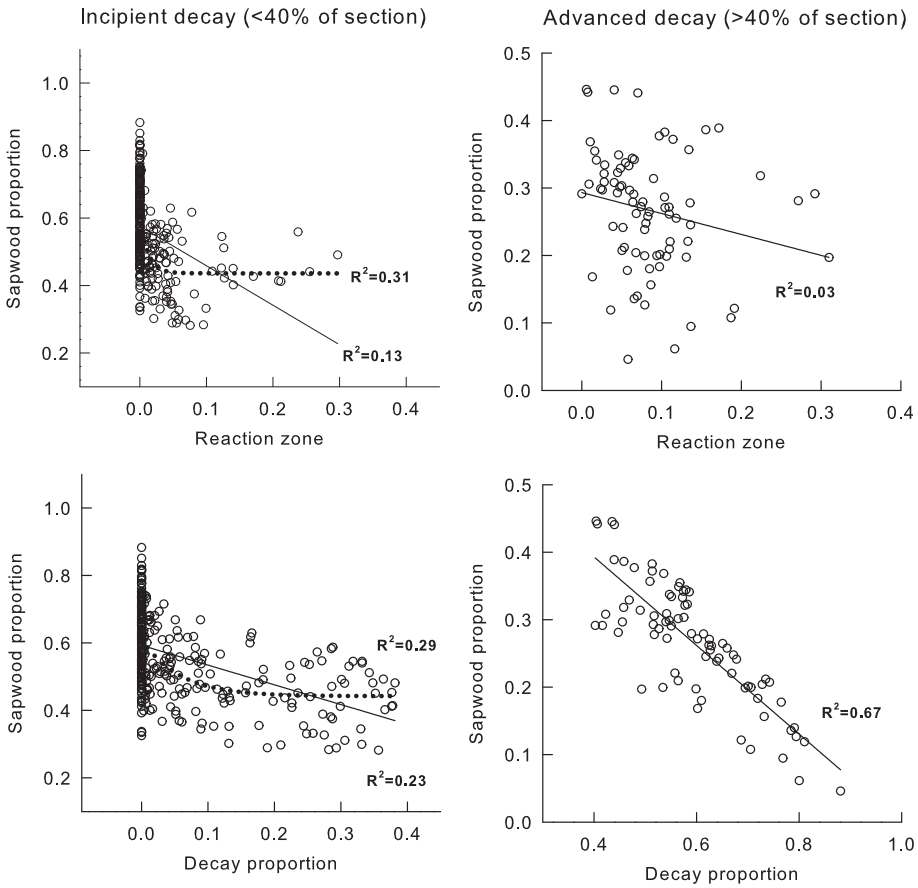

Fig. 3. Reaction zone, decay and sapwood size patterns in trees with incipient decay (<40% of the section) and trees with advanced decay (>40% of the section). Adjusted R^2 is shown. In the case of incipient decay, a linear model (solid line) is compared with an exponential decay model (dotted line).

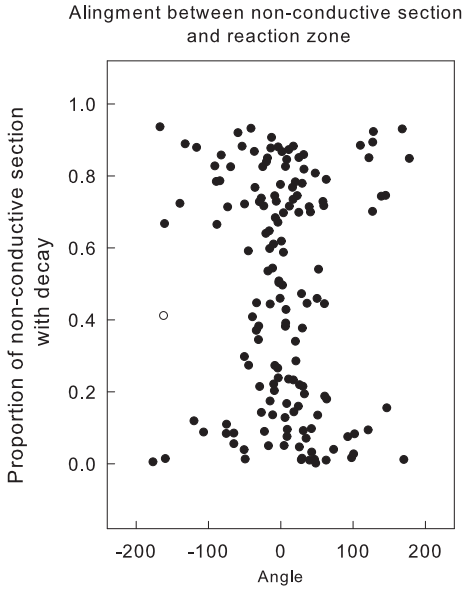

Fig. 4. Lateral shift of sapwood due to reaction zone formation. The lateral shift of the sapwood is consistent with the position of the reaction zone, when the decay column represents 20–60% of the non-conductive part of the section. When the decay column becomes larger (>60%), the reaction zone is present all around the decay column and the alignment tends to disappear. The angle between the centroid of the section and the centroid of the non-conductive part of the section and the reaction zone is used as indicative of the alignment (see Fig. 1). The tree represented as an open circle indicates a case in which decay occurred all over the border between the sapwood and the heartwood but a substantial part of the heartwood was not colonized.

sured (period 1997–2007), the growth rate of trees in the early stages of decay that had formed a reaction zone was more similar to that of trees with large decay columns (Fig. 5).

A larger sapwood was associated with a greater radial growth, although the correlation was moderate ($R^2 = 0.29$, $p < 0.0001$). Sapwood variation was better explained when trees were sorted by their reaction zone size ($R^2 = 0.57$, $p < 0.0001$). Within the same growth class, trees with a reaction zone tended to have smaller sapwood than trees without a reaction zone (Fig. 6).

3.3. Modelling sapwood, reaction zone and growth

We did not detect evidence of the direct effects of decay on tree growth, rather these effects were mediated by the creation of a reaction zone ($p = 0.004$) (Fig. 7b). When including sapwood in the analysis, SEM analysis supported the 'defence allocation' model as opposed to the 'sapwood reduction' model. According to the 'defence allocation' model, the tree would create a reaction zone, thus reducing its growth, which would result in a smaller proportion of sapwood (Fig. 7a). Decay did not directly cause a reduction in growth rate; instead the effect of decay seemed mediated by the creation of a reaction zone (Fig. 7a). According to the 'defence allocation' model (Fig. 7a), a smaller sapwood would result, directly, from decay formation ($p < 0.0001$) and, indirectly, from reaction zone formation by reducing tree growth ($p = 0.020$) (Fig. 7a).

The 'sapwood reduction' model was poorly supported by SEM (Fig. 7c). Sapwood size seemed better explained as a result of previous low growth than as the cause of reduced growth (Fig. 7d). Modifications to the 'sapwood reduction' model (Fig. 7d) were sug-

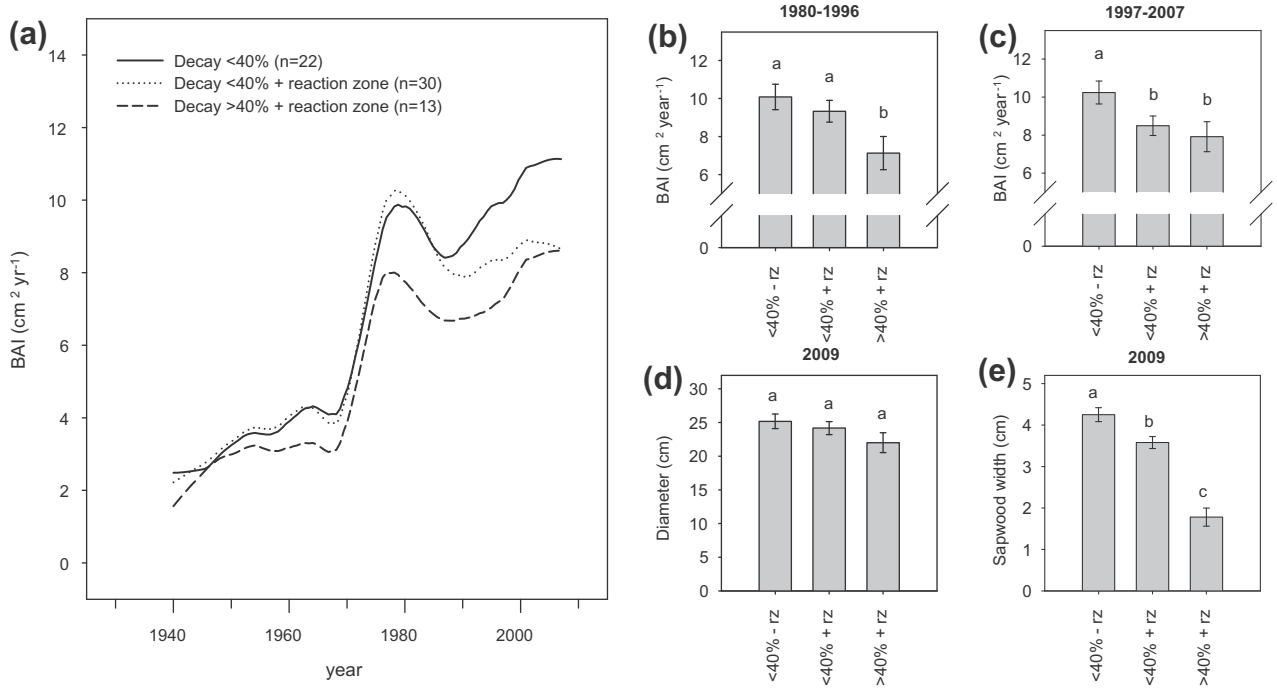

Fig. 5. (a)Trends in the basal area increment (BAI) of trees with small or no decay columns (<40% of the section) that did (<40% +rz) or did not (<40% −rz) develop a reaction zone, and trees with advanced decay columns (>40% of the section) (>40% +rz). Differences in mean BAI during the periods 1980–1996 and 1997–2007 (b and c, respectively), and for 2009, differences in diameter at breast height and sapwood width are also shown (d and e, respectively). Different letters imply significant differences at $p < 0.05$.

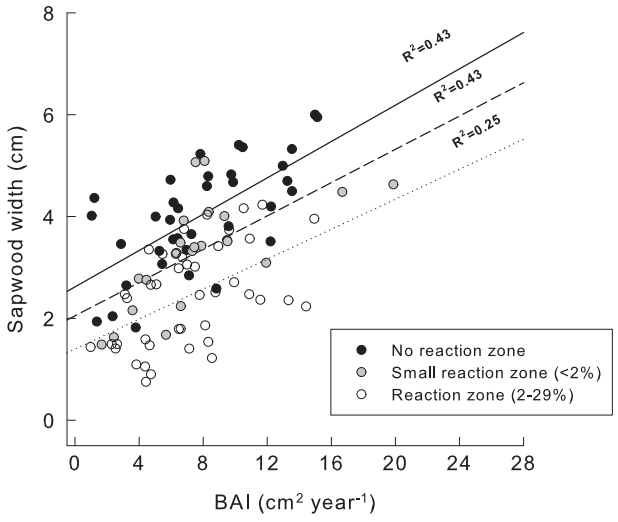

Fig. 6. Associations between sapwood width and radial growth (BAI, basal area increment), considering different sizes of reaction zone. The sapwood–BAI intercepts for the three sizes of reaction zone significantly differed ($p < 0.0001$). Growth represents the average BAI for the period from 1980 until 2007.

gested, but a significant fit to the data was only accomplished when basal area i.e. tree dominance was also associated with sapwood width. In that case, a positive association between growth and decay was encountered.

The 'defence allocation' model was tested independently at all three locations (Table 1) and produced a significant fit to the data (Ingbo, $p = 0.634$, Enåker, $p = 0.601$, Harsbo, $p = 0.709$). At all three locations, the negative influence of reaction zone on growth was supported ($p = 0.043$, $p = 0.018$ and $p = 0.035$). Given that decay is responsible for alterations in sapwood-width mainly at later stages of decay (Fig. 3), we tested the 'defence allocation' model at early stages of decay (<40% of the section). The model was highly significant ($p = 0.568$); however, the direct effect of decay on the proportion of sapwood was no longer supported ($p = 0.101$).

4. Discussion

The lack of strong constitutive defences in Norway spruce contrasts with its capacity to mount a strong defence response when under attack from pest and pathogens (Krokene et al., 1999; Deflorio et al., 2011). The capacity to mount a strong defence response must be coupled with an efficient system to allocate photosynthetic resources into secondary metabolite production. In our study, we provide evidence that allocating resources for defence against wood decay fungi could have an impact on radial growth in Norway spruce, and that the shift from growth to defence occurs as soon as the trees detect the pathogen attacking the sapwood from within the tree. Previous studies showed that when the pathogenic fungus *H. annosum* was detected by the living cells of the sapwood, trees reacted by creating a reaction zone with fungistatic properties (Stenlid and Johansson, 1987). Accordingly, we observed that spruce trees at initial stages of decay did not show a reduction in radial growth when the decay developed in the heartwood (Fig. 5). This observation corresponds well with the fact that a defence response could not be elicited owing the absence of living cells. In the studied trees, it was not possible to know exactly when the fungus spread from heartwood to sapwood. However, by comparing the growth pattern of trees along a decay development gradient, we did observe that growth losses occurred on trees at early stages of decay, presumably, when the first reaction zone was formed. In our study, two types of trees with a reaction zone were compared from 1980 until 2007: trees with small decay columns and trees with large decay columns. Although trees with small decay columns grew more than trees with large decay columns in 1980, the growth trend shifted and by 2007 these trees were growing at the same rate despite having decay columns that were less than a quarter of the size of the large decay columns (12% versus

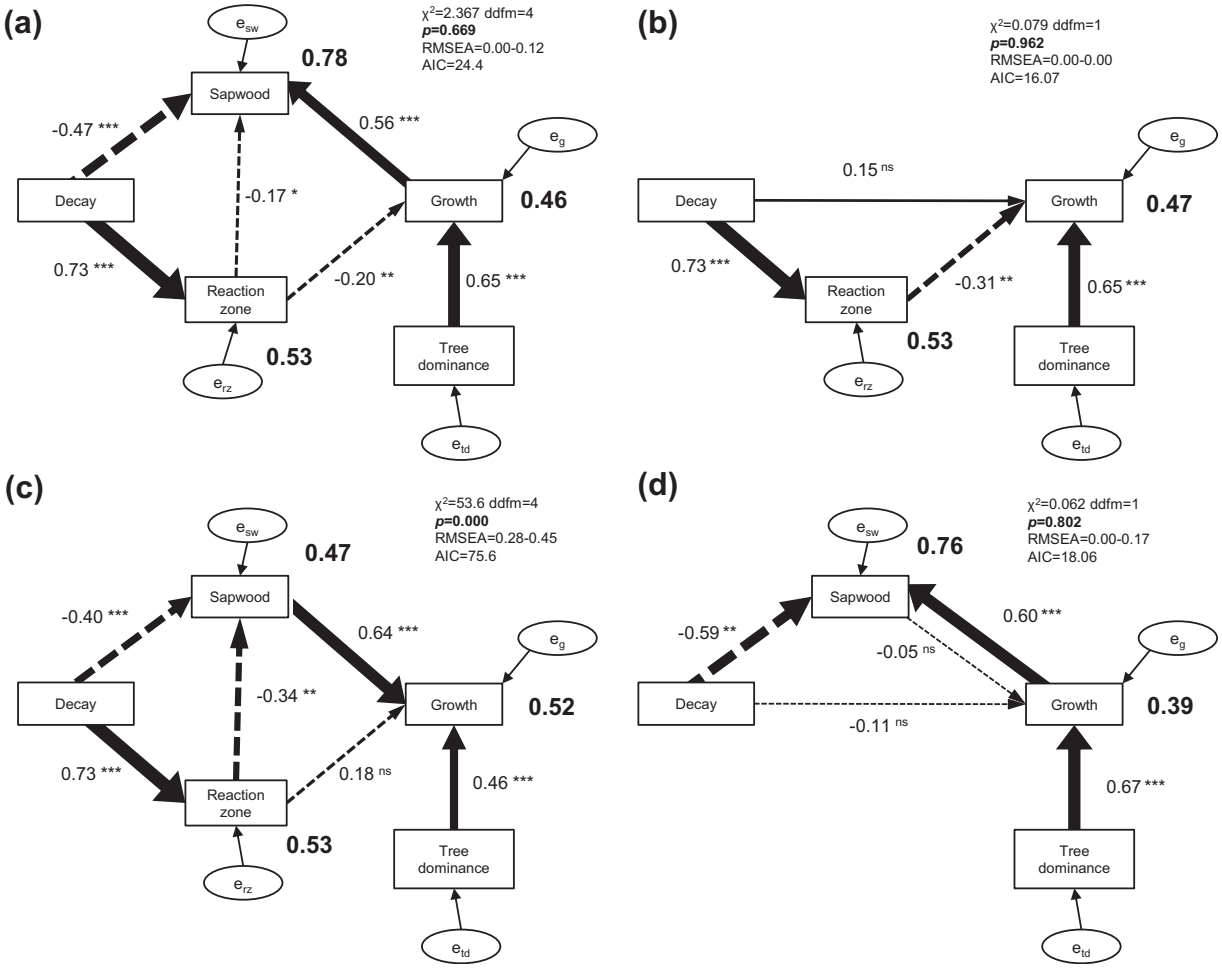

Fig. 7. Structural equation model (SEM) analysis of postulated mechanisms of influence of decay, reaction zone formation and sapwood on radial growth depending on their dominance in the canopy (n = 100 trees). Two pathways of carbon allocation were evaluated: (i) decay creates a reaction zone and reduces growth, shown in models (a) and (b), and (ii) decay reduces sapwood and thus reduces growth, shown in models (c) and (d). Sapwood is quantified as the sapwood width at 1.30 m, the reaction zone was the proportion of the section with reaction zone at 0.30 m, 0.66 m and 1.30 m, the growth was the mean basal area increment of the past 10 years, and the tree dominance was expressed as the basal area of the tree 10 years ago. The error terms of every dependent variable are represented in ellipses. The root mean square error (RMSEA) is expressed on its lower and upper limits at 95% and the degrees of freedom of the model (ddfm) are indicated after the χ^2 statistic. The Akaike information criterion (AIC) of each model is shown. The R^2 of each variable is shown in bold face. Significant levels of the standardized path coefficients: ns, not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

57%). This was not the case for trees without a reaction zone because they kept growing at a higher rate throughout the whole period (1980–2007). SEM analysis indicated that the transition from a normal growth rate towards a reduced growth rate was mediated by the creation of a reaction zone rather than by the size of the decay. Similar results were found in the case of *Armillaria* root rot on interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) by Cruickshank et al. (2011). In their study, growth losses due to defence were barely dependent on the amount of infection (% of root system infected). Rather, growth reduction was mostly dependent on the amount of time since the tree had been infected, and thus defence responses had been elicited.

Sapwood reduction did not seem to be a cause of growth reduction but rather to be the consequence of previously reduced radial growth. Assuming that sapwood area is well associated with crown area (Sellin, 1994), and also with the root system (Gould and Harrington, 2008), our results did not support a big effect of root damage or reduced sapwood on tree growth (Kelsey et al., 1998). As previously observed in an earlier study by Sellin (1994), sapwood size in our study appeared to be poorly correlated with tree growth, supporting the idea that conifers can develop normally even with a severely reduced conductive area (Zimmermann, 1983). As mentioned earlier, our results support the findings by Cruickshank et al. (2011) who showed that growth reduction in *Armillaria*-infected trees was poorly correlated with the amount of root system infected, and was dependent on the time since tree defences were first elicited. However, in our study the roots were not inspected, thus an effect of root damage could not be totally discarded. Contrary to our 'sapwood reduction' hypothesis, SEM modelling strongly supported the idea that the creation of a reaction zone had a significant effect on the proportion of sapwood area recorded during a period of low growth. In line with our results, the application of the phytohormone methyl jasmonate to Norway spruce trees (Krokene et al., 2008) reduced the sapwood growth rate by 30% and induced similar defence reactions to those induced by a fungal attack. According to our results, a poor crown condition, which is often observed in trees with butt rot (Vollbrecht and Agestam, 1995; Omdal et al., 2004), may partly reflect the readjustment of the crown to sapwood area losses as a result of periods of previous low radial growth.

The reaction zone did not seem to affect tree radial growth by physically replacing sapwood area. In our study, decay columns often appeared to be associated with the border of the sapwood. *Heterobasidion annosum* s.l. was the causal agent of most of the decay observed in this study. The majority of the fungal biomass was found just in front of the reaction zone by Hietala et al. (2009),

revealing the natural tendency of the fungus to spread towards the outer part of the tree. According to our observations, at intermediate stages of decay, this translated into sapwood eccentricity, suggesting that the local reduction in the rate of sapwood growth is compensated for by a greater sapwood width in the opposite direction. However, even in these cases, trees with a reaction zone were consistently found to have smaller areas of sapwood than trees without a reaction zone (Fig. 3). A rapid decrease in sapwood area soon after the first reaction zone is created supports a qualitative shift in the allocation of tree resources towards defence when stress factors appear.

Carbon allocation towards defence seems to be supported by previous studies at the cellular level. *Heterobasidion* secretes several enzymes to detoxify, degrade and kill host cells (Asiegbu et al., 1998). The host reacts by *de novo* production of several chemical and morphological responses such as the production of resin and phenolic compounds, lignification and suberization, and also by producing an array of pathogenicity related proteins such as chitinases, glucanases and peroxidases (Asiegbu et al., 1998). Several of these defence responses imply an increase in the *de novo* production of enzymes catalysing the first steps of the phenyl-propanoid pathway (Adomas et al., 2007; Koutaniemi et al., 2007). Unfortunately, little is known about the molecular responses of the sapwood; however, a recent study on Sitka spruce (*Picea sitchensis*) revealed a much slower activation of the phenyl-propanoid pathway in the sapwood than in the bark in response to wounding and *H. annosum* inoculation (Deflorio et al., 2011). Although bark infections and sapwood decay may not elicit the same responses, a slower response in the sapwood seems consistent with the long time needed to observe decay-induced growth reductions in this and previous studies (Bendz-Hellgren and Stenlid, 1995, 1997; Oliva et al., 2010), as compared with bark infection (Krokene et al., 2008).

The exact mechanism of formation of the reaction zone is not yet understood (Hietala et al., 2009) but its higher density than the sapwood (Oliva et al., 2011) supports the idea of an allocation of carbon from other parts of the sapwood. Although not all decay could be ascribed to *Heterobasidion*, we would not expect that pathogen-specific patterns would affect our observations, since reaction zone is considered to be an un-specific response. Supporting this fact we could see how our 'defence allocation model' fit data from Harbso and Enåker equally well, although with apparent differences in terms of *Heterobasidion* isolation rate. The production of the reaction zone may not only act as a carbon sink itself but it might also trigger responses in other distal parts of the tree further increasing carbon allocation towards a defence response. Systemic induced resistance has been observed in four-year-old Norway spruce saplings when challenged by *H. annosum* (Swedjemark et al., 2007). Induced resistance in Norway spruce is probably not pathogen specific (Krokene et al., 1999) and not restricted to the point of infection, given that Christiansen et al. (1999) observed that the activated defence mechanisms of the tree extended a few metres beyond the point of infection. Indeed, when we observed decayed trees without a reaction zone at stump height, they might have been hiding decayed roots with a reaction zone that should also have triggered defence responses. However, Christiansen et al. (1999) observed that an induced defence response in Norway spruce was dose-dependent in the case of *Ophiostoma polonicum*; therefore a sufficient amount of reaction zone may be needed to shift towards a systemic induced defence response. Indeed, the timing and the amount of the systemic induced response is probably under genetic control (Deflorio et al., 2011), as shown by Wallis et al. (2008) in Austrian pine (*Pinus nigra*).

Tree growth losses in response to *H. annosum* attacks have been associated with reaction zone formation in previous large-scale studies (Oliva et al., 2010). To the best of our knowledge, this is the first study in which growth reduction at different decay and reaction zone development stages has been studied. However, there is a need for caution in any attempt to use non-sequential data in order to draw sequential conclusions, thus our findings should be experimentally confirmed in later studies. Bendz-Hellgren and Stenlid (1995) hypothesized that the energetic cost of reaction zone production could explain 50% of the growth reduction observed in decayed trees. Our results support the idea that resources are allocated away from radial growth to tree defence, which may be of interest for future tree breeding strategies against *H. annosum*. The capacity to exclude *H. annosum* from Norway spruce has been found to be clone-dependent (Swedjemark et al., 2007), which, together with our results, suggest the possibility of a trade-off between growth and defence. Trees resistant to *Ophiostoma polonicum* infection have been shown to produce flavonoids more rapidly than the susceptible clones (Brignolas et al., 1995), possibly carrying over a relatively higher expression of the phenyl propanoid pathway and, therefore, a higher allocation of carbon towards defence. If a similar response is pursued in the case of *H. annosum*-resistant clones, breeders may want to balance the costs and benefits of selecting either *H. annosum*-tolerant genotypes at the expense of increased losses due to high volumes of decayed wood or very resistant trees that may have smaller decay columns but at the expense of lower growth rates.

Acknowledgements

We are thankful to Stora Enso who provided the plots for the experiment. Carmen Romeralo and Heriberto Vélëz contributed to the field work. This research was partially funded by the project 'Future Forests', financed by the MISTRA Foundation for Strategic Research. Jonàs Oliva was funded by the post-doctoral grant 2008 BP A 00013 from the DURSI-GENCAT. J. Julio Camarero thanks the support of ARAID. We thank G. Sanguesa-Barreda for measuring tree rings. Comments and suggestions by two anonymous referees are greatly appreciated.

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