

Costly post-dieback recovery: winter drought impairs xylem phenology, anatomy and growth in Mediterranean Scots pine forests

Authors: J. Julio Camarero¹, Guillermo Guada², Raúl Sánchez-Salguero³ and Emilio Cervantes⁴

Affiliations:

¹Instituto Pirenaico de Ecología (IPE-CSIC). Avda. Montañana 1005, 50192 Zaragoza, Spain.

²Departamento de Botánica, EPS, Campus de Lugo, Univ. Santiago de Compostela, 27002 Lugo, Spain.

³Departamento de Sistemas Físicos, Químicos y Naturales, Universidad Pablo de Olavide, Sevilla, Spain.

⁴Departamento de Producción Vegetal, IRNASA-CSIC. Apartado 257, Salamanca, Spain.

Corresponding author:

Jesús Julio Camarero

Instituto Pirenaico de Ecología, IPE-CSIC

Avda. Montañana 1005, 50080 Zaragoza, Spain.

Tel.: +34 976 716031 / Fax: +34 976 716019

E-mail: jjcamarero@ipe.csic.es

Abstract

Continental Mediterranean forests face drought and cold stress. Drought and cold spells are climate extremes which can impair the recovery and resilience capacity of some Mediterranean forests. Climate warming could amplify the negative effects on forests of climate extremes by altering phenology phases (premature dehardening) and by magnifying drought stress. Here we capitalize on a winter-drought induced dieback triggered by a cold spell which occurred in December 2001 and affected Scots pine forests in E. Spain. We assessed post-dieback recovery by quantifying and comparing radial growth and xylem anatomy of non-declining (crown cover > 50%) and declining (crown cover \leq 50%) trees in two sites (VP, Villarroya de los Pinares; TO, Torrijas). We also characterized aboveground productivity in site VP and xylogenesis in both sites during 2005. Dieback caused legacy effects since needle loss, a 60% reduction in aboveground biomass and radial-growth decline characterized declining trees three years after these dieback symptoms started in spring 2002. Declining trees formed collapsed tracheids in the 2002-ring earlywood, particularly in the most affected VP site where xylogenesis differences between non-declining and declining trees were most noticeable. The lower growth rates of declining trees were explained by a shorter duration of their major xylogenesis phases. In the site VP the radial-enlargement and wall-thickening of tracheids were significantly reduced in declining trees as compared to non-declining trees, and this was because these phases tended to start earlier and end later in non-declining than in declining trees. Gompertz models fitted to tracheid production predicted that maximum growth rates occurred 11-12 days earlier in non-declining than in declining trees. The formation of radially-enlarging tracheids was enhanced by longer days in both study sites and also by wetter conditions in the driest TO site, but this xylogenesis sensitivity to climate was reduced in declining trees. Winter-drought induced dieback impairs xylem anatomy and phenology, aboveground productivity, xylogenesis and growth in Mediterranean Scots pine populations. Affected stands show a costly post-dieback recovery challenging their resilience ability.

Key words: cambium, climatic extremes, dendroecology, needle loss, *Pinus sylvestris*, resilience, xylogenesis.

Introduction

Mediterranean conifer forests face a high climatic variability, particularly regarding water availability, but they usually recover following adverse climatic conditions such as droughts (Camarero *et al.*, 2015b). The recent warmer conditions, when superimposed on severe water deficit, have caused growth decline, vigour loss and dieback in some Mediterranean pine forests (e.g., Sánchez-Salguero *et al.*, 2012). Such intensified drought impact challenges the recovery capacity of some of these forests but we still do not know how forest growth and productivity recover after these harsh climate events, and how the recovery component of resilience is impaired by similar rare and unpredictable climate extremes.

Severe droughts illustrate the relevance of climate extremes, an often underestimated component of climate change affecting the resilience capacity of Mediterranean forests (Smith 2011). However, inland Mediterranean forests subjected to continental conditions face other climate extremes than droughts such as cold spells (Camarero *et al.* 2015a). It has been argued that climate warming could increase the risk of cold-induced forest dieback if mild conditions induce phenological alterations (e.g., late hardening) making tree tissues more vulnerable to low temperatures (Leinonen, 1996). Therefore, phenological timing influences how sensitive are tissues to rapid drops in air and soil temperatures influencing the final tree damage (Sakai and Larcher, 1987). Such cold-induced damage involves xylem embolism induced by freeze-thaw cycles, cell dehydration and membrane breakage (Pearce, 2001; Mayr *et al.* 2003).

Research on cold-induced dieback is biased towards boreal and subalpine conifer forests, unsurprisingly (Kullman 1989; Lazarus *et al.* 2004). Nonetheless, this dieback type is not restricted to cold biomes since it has been described in dry and continental areas including Mediterranean-type forests (Soulé and Knapp, 2007; Matusick *et al.*, 2014; Camarero *et al.* 2015a). Here we capitalize on a winter-drought induced dieback which was driven by a cold spell occurring in winter 2001-2002 (Voltas *et al.* 2013). This dieback episode affected ca. 14,000 ha of Mediterranean Scots pine forests situated in the Spanish Sistema Ibérico range,

near the southernmost distribution limit of the species distribution area (Fig. 1). Several field observations performed by the authors since spring 2002 agree with winter-drought symptoms (Camarero and Sancho-Benages 2006). First, dieback (needle shedding, bud and shoot death, growth decline, increased mortality) was most prevalent in south-oriented sites located at mid elevations, i.e. with shallow or absent snow pack, whose soils are thin and rocky thus having a low capacity to store water. Second, needle loss was also more important in the south-facing side of crowns of relatively tall trees subjected to the highest air thermal contrast (by contrast, shrubby junipers were not affected; see Fig. 1a). In addition, affected trees showed pronounced xylem embolism which was attributed to freeze-thaw cycles (Peguero-Pina et al. 2011).

Since the rarity of climate extremes such as the winter 2001-2002 cold spell makes difficult documenting their impacts on forest resilience here we follow a retrospective approach by reconstructing radial growth of trees differently affected using dendrochronology (Dobbertin, 2005). We use needle loss (crown cover) as a proxy of tree vitality to differentiate two types of coexisting Scots pine trees showing contrasting dieback intensity in response to the winter-drought event. We characterize xylogenesis (phenology of xylem development) in these two classes of trees three years after the dieback symptoms started in spring 2002. Lastly, we also quantify aboveground productivity in one more intensively monitored site, and describe alterations in xylem anatomy. The combined use of growth and xylogenesis data constitutes a valuable tool to evaluate how forest resilience of these Scots pine stands was impaired by winter-drought dieback, a poorly characterized phenomenon affecting Mediterranean forests subjected to continental conditions. Our specific objectives were: (i) to quantify the radial-growth responses to the 2001-2002 cold spell; (ii) and to determine if the winter-drought induced dieback altered xylem anatomy and xylogenesis. Lastly, we aim to demonstrate how extreme cold events negatively impact forest resilience in unexpected places as the southern distribution limit of a widely distributed tree species as Scots pine.

Material and methods

Study area

The study was carried out in two sites (Villarroya de los Pinares, 40° 30' 21'' N, 00° 37' 03'' W, 1670 m a.s.l.; hereafter abbreviated as VP site; Torrijas, 39° 59' 21''N, 00° 54' 28'' W, 1600 m; hereafter abbreviated as TO site). These sites are located in the Scots pine forested area affected by the winter-drought dieback and they are situated in the Gúdar (VP site) and Javalambre (TO site) ranges, Spanish Sistema Ibérico range, Teruel province (Fig. 1). Both sites presented N-NE exposure and were located on relatively flat drainage areas extending about 2 km² (slopes were 5° and 15° in VP and TO sites, respectively). In this area, Scots pine (*Pinus sylvestris* L.) forms pure or mixed pine forests with the understory dominated by juniper (*Juniperus communis* L.) and shrub species (mainly *Berberis vulgaris* L.). In a similarly affected stand located near the VP site, a previous study reported mean values of 10.4 m² ha⁻¹ and 348 stems ha⁻¹ for basal area and density, respectively (Camarero and Sancho-Benages 2006). These features correspond to relatively open stands where the intensity of competition is expected to be low. Soils are usually shallow and rocky and developed on calcareous outcrops.

These sites experience a continental Mediterranean climate characterized by cold winters (mean temperatures vary from 1.6° to 4.0 °C, absolute minimum temperatures usually range between -4.0° and -21.0 °C) and dry and warm summers (mean total precipitation varies from 124 to 140 mm, mean temperatures fluctuate from 14.3° to 20.7 °C, absolute maximum temperatures range between 21.0 ° and 35.0 °C). The annual precipitation in the study area is ca. 650 mm. According to data from nearby stations (located at ca. 7 km from the study sites) site VP is colder (mean temperature 9.0 °C) than site TO (mean temperature 10.4 °C) (Supporting Information, Fig. S1).

Climate data

To obtain *in situ* climate data, the air temperature and relative humidity were measured by using two data loggers (Hobo H8 Pro Series, Onset, Bourne, USA) per site located in a tree placed in the north- and south-oriented sides of the stem and at 4 m above the ground (Supporting Information, Fig. S2). Climatic data were recorded every 30 minutes and converted to daily values either by averaging (temperature) or by summing (precipitation) them. Daily rainfall data were obtained from nearby meteorological stations (site VP, station Villarroya de los Pinares, 40° 31' N, 0° 40' W, 1337 m; site TO, station Torrijas-Los Cerezos, 40° 01' N, 0° 57' W, 1356 m). We also calculated the daily water balance as the difference between precipitation and the estimated potential evapotranspiration (Hargreaves and Samani 1982). Lastly, data on day length (photoperiod) were obtained from the Teruel climatic station (40° 21' N, 1° 07' W, 900 m), located at 40 km from the study sites.

Previous climate conditions during winter 2001-2002

The winter 2001-2002 was preceded by warm fall conditions (the mean October 2001 temperature represented a +2.0° C anomaly; cf. Voltas et al. 2013). Conditions in December 2001 were relatively dry (41% and 58% of the average in VP and TO sites, respectively) and very cold because a sharp drop of temperatures occurred in the second half of this month. The absolute minimum December temperatures recorded in Teruel station (-22° C) were the lowest recorded for that month since the late 19th century (Camarero et al. 2015a). The monitoring year (2005) was characterized by relatively dry spring conditions, particularly in the TO site where precipitation was 31% of the average whilst it was 48% in the VP site (see Supporting Information, Fig. S2).

Field sampling

In each location ten declining and ten non-declining adult Scots pine trees were randomly selected, sampled and measured (Dbh, diameter at breast height measured at 1.3 m; tree height,

horizontal projection of the crown diameter). For each sampled tree, the proportion of crown cover was estimated to the nearest 5% (Müller and Stierlin, 1990). Since estimates of crown cover vary among observers and forests, the data were always recorded by the first author, who compared every tree with a reference tree with the maximum amount of foliage at each site. Non-declining trees were considered those with a crown cover of > 50% and declining trees those with a crown cover of \leq 50% (note that separations based on other crown-cover thresholds such as 40% and 60% rendered similar results; cf. Camarero et al., 2015b).

Estimates of primary growth and aboveground productivity

To obtain an estimate of the impact of the dieback on aboveground productivity we placed 4 traps under each of the trees sampled in the VP site. This site was selected for this intensive monitoring because it was located in the most extensive affected area (Fig. 1) and declining trees presented the lowest crown cover (Table 1). Traps were cylindrical containers of 0.014 m² collecting surface coated with fine mesh bags and placed 0.5 m above the ground at 1.5 m from the main stem, i.e. always under the crown projection. Each trap was placed in four opposite directions with respect to the stem (SW, NE, SE and NW). Samples were monthly collected from traps and from May 2005 to May 2006. Since we did not find significant differences between declining and non-declining trees considering monthly data (ANOVAs, $P > 0.05$), data were summed and presented as yearly values of dry biomass (g m⁻²). Total aboveground biomass was obtained, and we also measured separately the dry weight of the four major components (needles, bark, shoots and male cones). Collected material was oven-dried at 60° C until constant weight was reached (usually 5 days) for determining dry mass. Lastly, to measure several crown variables (shoot length, needle length, frequency of shoots with dead apical buds, and frequency shoots with male cones), six branches at least 10 years old were harvested per tree in two different positions (three sunny branches located at the top of the canopy, three shaded branches located at the mid canopy). In each branch, five shoots were

collected to measure their length and to determine the presence of dead apical buds and male cones. From each shoot, ten current-year needles were harvested and their lengths were measured to the nearest 0.1 mm. The branches were collected according to the phenological phase of interest, specifically: a first sampling was done in May 2005 to study male cone production, a second sampling was done in August 2005 to measure shoot and needle variables, and a third sampling was done in April 2006 to estimate bud viability. Dead buds were identified by their dry and hollow aspect and brown color. In addition, dead buds were usually shed and they were not viable since they did not lead to spring bud break.

Dendrochronological methods

Dendrochronological sampling was performed following standard methods (Fritts, 2001). Two cores were taken from each tree at 1.3 m using a Pressler increment borer. A third core was obtained near the base of the tree to estimate its age. The wood samples were air-dried and polished with a series of successively finer sand-paper grits. Then, the samples were visually cross-dated. Tree rings were measured to the nearest 0.01 mm using a binocular scope and a LINTAB measuring device (Rinntech, Heidelberg, Germany). Tree-ring cross-dating was checked using the program COFECHA (Holmes, 1983).

Since basal-area increment (BAI) is assumed to be a more meaningful indicator of tree growth than tree-ring width, ring widths were converted to BAI assuming a circular outline of stem cross-sections and using the formula:

$$\text{BAI} = \pi (R_t^2 - R_{t-1}^2) \quad (1)$$

where R is the radius of the tree and t is the year of tree ring formation. In healthy dominant trees, BAI series usually show an early suppression phase followed by a rapid increase and final stable phase. Mean annual values of BAI were separately obtained for declining and non-declining trees.

Xylogenesis

The cambium generates tracheids passing through different developmental stages and this process of xylem differentiation is called xylogenesis (Fukuda 1996). Xylogenesis was monitored in declining and non-declining trees by sampling wood micro-cores (2 mm in diameter, 1-2 cm in length) from January until December 2005. Sampling was done monthly in January and February, when growth in similar forests has not usually started (Camarero et al. 1998, 2010), and biweekly the rest of the year. In this analysis we sampled five trees per vigour class in both study sites. Samples were taken around the stems at 0.5-2.0 m using a Trephor increment puncher (Rossi et al., 2006a). The thick dead outer bark was removed, and sampling positions were arranged along an ascending spiral pattern in the stem (Deslauriers et al. 2003). The micro-cores were taken about 5 cm apart from each other to avoid wound reaction. The samples usually contained the tree rings corresponding to the 2002-2005 period.

In the field micro-cores were immediately placed in Eppendorf tubes containing a mixed solution of formaldehyde, acetic acid and ethanol (5:5:90) and stored as soon as possible at 5° C to avoid tissue deterioration. All samples were then processed within a maximum of 1-2 weeks after sampling. Micro-cores were sectioned using a sledge microtome (Anglia Scientific AS 2000, UK) achieving samples 20-µm thick. Sections were mounted on glass slides, stained with 0.5% water solution of cresyl fast violet, fixed with Eukitt® and observed at 100-200x magnification under a light microscope (Olympus BH2). Four different xylogenesis phases were identified following Wodzicki (1971) and Antonova and Stasova (1993): (1) cambial cells characterized by small radial diameters, thin walls and bone shape; (2) radially-enlarging tracheids presenting unlignified cell walls and therefore unstained in blue; (3) wall-thickening and lignified tracheids with a transition coloration from violet to dark; and (4) mature cells with lignified cells walls fully stained in blue. The numbers of cells in each of the four different phases were counted along five radial rows to obtain a mean value per ring and sampling date. The total number of tracheids formed during 2005 were also counted along five cell rows and

then averaged to obtain a relative number of 2005 tracheids as compared with the means of the previous three years. Finally, we selected some xylem cross-sections from VP trees presenting abnormal anatomical features (e.g. collapsed earlywood tracheids) in the 2002 tree ring to take pictures under a confocal microscope (Leica TCS-SPII) with an objective of 60 x (water immersion).

Timing of wood formation

Tracheid differentiation was considered to have started and to be complete when at least one horizontal row of cells was detected in the enlarging phase and cell wall thickening and lignification were completed, respectively (Gruber et al. 2010). To precisely define and compare xylogenesis between vigour classes we computed the onset and cessation dates and the duration of three developmental phases (E, radial enlargement; L, cell-wall thickening and lignifications; M, tracheid maturation) using the package CAVIAR (Rathgeber *et al.*, 2011) in the R statistical program (R Development Core Team, 2015). The onset and cessation dates were defined when 50% of the radial files were active (onset) or non-active (cessation) in each xylogenesis phase. The durations of each phase were calculated as the time elapsed between the onset and cessation of these phases. Xylem formation was defined as the time elapsed between the onset of enlargement and the end of maturation. Finally, to compare the onset and cessation dates and the duration of the main phases of xylogenesis we used the achieved significance level (ASL), which is analogously interpreted to a *P* significance level since the smaller the value of ASL, the stronger the evidence against the null hypothesis (Efron and Tibshirani 1993). In this case, the null hypothesis considers no difference between vigour classes regarding dates or durations of xylogenesis phases. ASLs were calculated based on 10000 bootstrapped iterations of the original data.

Statistical analyses

We used Mann-Whitney U tests to compare characteristics of trees between the two vigour classes. The rate of production of mature tracheids (tracheids day⁻¹) was obtained by subtracting the cumulative number of mature tracheids between consecutive dates and dividing this number by the number of days elapsed between those dates. The increase in the cumulative number of tracheids along time as a function of time (t) was modelled for each tree using the Gompertz function:

$$y = a \cdot e^{\left(-e^{b-ct}\right)} \quad (2),$$

which has been previously used in xylogenesis studies on Scots pine and other tree species (Camarero et al. 1998, Rossi et al. 2003). Time (t , in days) was considered to start ($t = 0$) the 7th April. The following parameters derived from the Gompertz function were obtained for each tree: t_p , date of the inflection point of the function; r , mean rate; and t_d , days required to attain most growth (Deslauriers et al. 2003, 2005).

The associations between climate and xylogenesis data (number of cambium cells, number of tracheids in different developmental stages, rate of mature tracheid production) were evaluated at 7 and 15-day long time scales since growth dynamics and responses to climate at similar time scales have been described in Scots pine (Antonova et al. 1995; Sánchez-Salguero et al. 2015). We used linear-mixed effects models to evaluate the effects of crown cover (vigour class) and climate variables on the ($x^{0.5}$ -transformed) number of different types of tracheids along time, and checked the predicted values and residuals looking for signals of heteroscedasticity (Zuur *et al.*, 2009). Time and vigour class were regarded as fixed factors, whereas tree was considered a random factor. These models were fitted using the *nlme* library (Pinheiro *et al.*, 2015). All statistical analyses were done using the R statistical program version 3.12 (R Development Core Team, 2015).

Results

Growth before and after the dieback event

In both sites, growth (BAI, annual number of tracheids) decreased after 2002 in the declining trees as compared with the non-declining trees was (Table 1, Fig. 2). In the declining trees the relative number of 2005 tracheids was on average 95%, whilst in the non-declining trees it was 58%. Prior to the dieback (1965-1997 period), declining trees grew more than non-declining trees in the VP site (Table 1).

Considering the data on aboveground biomass in trees from the VP site, the declining trees presented lower values than non-declining trees, and this was also observed for all measured components (needles, bark, shoots and male cones; Table 2). Declining trees also had more dead apical buds and a lower production of male cones than non-declining trees. In 2005, shoots were shorter in declining than in non-declining trees, but needles were longer (Table 2).

Xylem anatomy and xylogenesis

In 2005, a non-declining tree produced on average three times more tracheids than a non-declining tree (Table 1, Figs. 3a and b). The reduced growth capacity of these declining trees observed in 2005 followed the formation of abnormal xylem features such as the production of collapsed earlywood tracheids in the 2002 tree ring, particularly in the most affected VP site (Figs. 3c, 3d and 3e).

Declining trees produced less radially-enlarging and wall-thickening tracheids than non-declining trees and differences were larger in the VP than in the TO site (Fig. 4). Non-declining trees produced four to two times more mature tracheids per tree ring than declining trees in the VP and TO sites, respectively (Table 3). Consequently, the rate of production of mature tracheids also decreased in declining trees (Table 3).

Differences in timing of wood formation between declining and non-declining trees

The number of cambial cells started increasing in April and May in sites TO and VP, respectively (Fig. 4). Radially-enlarging and wall-thickening tracheids peaked from May to June and from June to August, respectively. Therefore, most growth occurred from April to August (Fig. 4). Regarding the onset and cessation dates and the duration of the main phases of tracheid differentiation, we detected differences between declining and non-declining trees in most of these phases in the VP site excepting the onset of tracheid radial enlargement (Fig. 5, Table 4). In the TO site differences were only detected concerning the duration of the wall-thickening and xylem-formation phases. Overall, differences in the timing of wood formation were more noticeable in the VP site. There, the radial-enlargement and wall-thickening phases started earlier and ended later in non-declining than in declining trees leading to longer phases of xylem development and thus wider tree rings in non-declining trees (Fig. 5).

Climatic influences on xylogenesis of declining and non-declining trees

Gompertz models were in agreement with xylogenesis observations and produced estimates of maximum tracheid-production rates four (site VP) to two (site TO) times higher in non-declining than in declining trees (Fig. 6). In addition, they estimated a lag of maximum-growth rates between the TO (mid May) and VP (early June) sites. More importantly, these models predicted that the maximum growth rates, occurring from May to June, were observed 11-12 days earlier in non-declining than in declining trees (Table 3, Fig. 6).

Linear mixed-effects models showed that the production of radially-enlarging tracheids was positively linked to weekly values of day length in both study sites (Table 5, Fig. 6). Wetter conditions at 15-day long scales also favored the production of radially-enlarging tracheids in site TO. The production rate of mature tracheids was also positively related to day length but again at 15-day long scales. Warmer night-time temperatures were associated to an increase in the number of wall-thickening and lignifying tracheids. Lastly, these models also indicated that xylogenesis of non-declining trees responded more to climate than in the case of

declining trees excepting cambial cells, which did not show clear associations to climate and presented the lowest percentages of explained variance (18-20%). In contrast, models of the production rate of mature tracheids rendered the highest percentages of explained variance (78-89%).

Discussion

Here we show that the extreme cold spell of winter 2001-2002 lead to winter-drought dieback and negatively impacted xylem growth, anatomy and xylogenesis. This case illustrates how an extreme climatic event impaired Scots pine forests subjected to Mediterranean and continental conditions thus compromising their resilience capacity. We present evidence that this dieback event altered xylem anatomy and produced collapsed tracheids in spring 2002. The sharp drop in temperatures during December 2001 was preceded by warm fall conditions which could have altered the hardening of some tissues, and freeze-thaw events during that relatively dry winter possibly caused xylem embolism (Peguero-Pina et al. 2011). The production of collapsed earlywood tracheids could be related to xylem cavitation.

Here we present evidence on legacy effects since the dieback started in 2002 but the growth reduction was still intense in 2005. The commented legacy effects were still apparent in winter 2009 when the average standing tree mortality at the most intensively monitored VP site was still about 10%, whereas ca. 47% of trees showed crown cover lower than 60% (Voltas et al. 2013). In the site VP, aboveground productivity of declining trees was clearly reduced according to trap data. The aboveground biomass was reduced by 60%, shoots were six times shorter on average in declining trees as compared with non-declining trees, and the frequency of dead buds greatly increased in declining trees (Table 2). This agrees with findings from French Mediterranean Scots pine forests where a severe spring drought negatively affected radial growth but also reduced crown density and the formation of female cones, albeit the production of male flowers was enhanced (Thabeet et al. 2009). Unexpectedly, needles from

declining trees showing a low crown cover were longer than those of non-declining trees and the presence of abnormal 3-fascicle needles was also observed in the affected trees (J.J. Camarero, *pers. observ.*). Usually, drought leads to the production of shorter and thinner needles in pine species growing in Mediterranean environments (Cinnirella et al. 2002), probably because of a decrease in cell elongation rates due to turgor losses (Larcher 1994). We speculate that the low crown cover values observed in 2005 among declining trees from site VP (Table 1) was also linked to altered development patterns which caused the formation of abnormally long needles which probably experienced less shading. For example, sun needles magnified drought effects on needle anatomy as compared with shade needles (Gebauer et al. 2015).

Drought stress caused by a reduced soil moisture limits radial enlargement of tracheids in conifers leading to the formation of abnormal tracheids with concaved tangential walls (Glerum 1970). Collapsed tracheids with thin walls showing secondary thickening but reduced lignification are also characteristic of drought-exposed trees (Barnett 1976). It is assumed that the more severe the drought, the more severe is the ensuing cell radial distortion due to a subsequent lowering of turgor in the expanding and differentiating tracheids causing either their collapse or distortion and probably terminating their differentiation (Larson 1994). It must be taken into account that drought can reduce growth rates through impairment of cell division and expansion, processes which happen at lower stress thresholds than photosynthesis inhibition (Hsiao et al. 1976). In the case of frost rings, one or several rows of collapsed tracheids with poorly developed walls are formed due to growing-season intense frosts that disrupt the xylem anatomy leading also to the formation of distorted rays and callus-like parenchymatous tissue (Glock 1951, Glerum and Farrar 1966). The abnormal tracheids described here (Fig. 3) resemble some of the anatomical features of frost rings since collapsed tracheids were formed in the early earlywood. However, frost events were not observed in the study area during 2002 early spring when growth started so they could not directly cause frost

injury even if the cambium is a tissue vulnerable to frost damage during active growth (Voltas et al. 2013; Camarero et al 2015a). Furthermore, the affected individuals were not young trees with thin barks, two features which predispose to frost-ring formation (Larson 1994). Schweingruber (2007) suggested that frost damage at the beginning of the ring could also indicate an extremely winter cold condition previous to cambial resumption in spring and this could be a plausible explanation for the pattern here described. High cambial temperatures during the day followed by a rapid fall to freezing night temperatures are also often associated to frost damage and injury to the cambium of trunks (Larson 1994). We propose that this is the probable mechanism which caused the xylem alterations we observed in the 2002 tree ring since warm and sunny conditions from October to December 2001 probably caused a premature dehardening of cambial tissues prior to the sharp drop of temperatures in late December which induced the dieback observed in spring 2002. Finally, the relatively dry 2005 spring could have further exacerbated the growth reduction of declining trees (Figs. 2, 3a, 3b and 4), but such dry spell was not related to the abnormal xylem anatomy observed in 2002 rings of declining trees (Figs. 3c-3e).

Declining trees grew less than non-declining trees in 2005, three years after the dieback started (Fig. 2). Remarkably, this difference was linked to a lagged start and a premature termination of the radial-enlargement and wall-thickening phases in the declining trees which consequently presented a shorter phase of xylem development than non-declining trees, particularly in the most affected VP site. Therefore, xylogenesis was altered in declining trees still presenting high defoliation and this explains why they showed lower growth rates (Table 3, Fig. 6). Such alterations in xylem development constitute legacy effects rarely described in the literature concerning forest dieback episodes. However, in declining silver fir (*Abies alba*) trees their stem wood experienced alteration in lignifications processes (Shortle and Ostrofsky, 1983) due to a premature end of wood formation characterized by an earlier differentiation of the latewood cell walls as compared with non-declining trees (Torelli et al., 1999).

How drought affects xylogenesis changes along the growing season and depends on the development phase and the stress due to water shortage. For instance, water deficit during the late growing season has been shown to induce an earlier cessation of cell division (Eilmann et al. 2011), but drought during the early growing season reduced the cross-sectional area of vessels in poplar (Arend and Fromm 2007). Several studies about the drought effects on xylogenesis of Scots pine found that stressed or defoliated trees formed narrow rings as the result of a shorter growing season mainly because of a premature cessation of cambial activity (Eilmann et al. 2011, 2013). Under mesic conditions the onset of cambial activity is largely controlled by temperature in Scots pine (Wodzicki 1971, Antonova and Stasova 1993), but drought has been shown to reduce the period of xylem differentiation by affecting the end of the growing season (Gruber et al. 2010, Swidrak et al. 2011; Fernández-de-Uña et al. 2013) as aforementioned in the case of the silver fir. In this study differences between vigour classes appeared regarding onset, cessation and duration of the major xylogenesis phases, and they were well reflected by Gompertz models (Figs. 4 and 5, Table 4). The duration of xylogenesis phases as the radial enlargement of tracheids is critical since it determines to a great extent the annual ring width (Cuny et al., 2014) and also the final transversal size of tracheids which depends on reaching an adequate turgor pressure during their expansion (Abe et al. 2003). This agrees with a recent study on Scots pine recovery after a drought-induced dieback showing that xylem formation started ca. 1 month earlier in the non-declining than in the declining and most defoliated trees which presented the shortest duration of the radial-enlargement phase (Guada et al. 2016).

Based on a dual C-O isotope approach and also using tree-ring width data, it was suggested that growth and intrinsic water-use efficiency of declining trees were more coupled with climate prior to the dieback than in the case of non-declining trees (Voltas et al. 2013). This was associated by a greater aptitude of declining trees to grow more and take up more water than non-declining trees making the former more vulnerable to hydraulic failure than the

latter. Therefore, declining trees probably exhibited a greater cavitation risk by forming tracheids with more ample lumen areas (Peguero-Pina et al. 2011). The higher growth rates of declining trees prior to the dieback event agree with what we observed in site VP but not in site TO (Fig. 2, Table 1). In consequence, the hypothesis of a high xylem cavitation risk as a predisposing factor of winter-drought induces dieback can be assumed in the cold-mesic site VP but not in the warm-dry site TO where drought during the growing season could also contribute to induce growth decline. Analyses of climate-xylogenesis associations confirmed this assertion since wetter conditions favored the formation of radially-enlarging tracheids in this site which was particularly sensitive to water availability (Table 5). In drought-prone sites, often characterized by a low ability to hold soil water (e.g. steep slopes, southern exposures, rocky substrates, shallow soils, sandy textures), Scots pine radial growth is constrained by dry spring and summer conditions during the growth year and also during the previous year and these constraints probably are very important in the site TO (Oberhuber et al. 1998; Sánchez-Salguero et al. 2015). Thus, the 2001 cold spell exposed the growth and physiological differences of co-occurring trees to winter-drought dieback, at least in the coldest VP site, and this occurred irrespective of the trees' ability to store carbohydrates (Voltas et al. 2013).

Conclude

We detected legacy effects (growth decline, low crown cover, reduced aboveground biomass) of a winter-drought induced dieback three years after needle shedding and growth decline started in two Scots pine forests. Declining trees formed collapsed earlywood tracheids the spring after a winter cold spell triggered the dieback. Declining trees also showed the lowest crown cover and radial-growth rates because their xylogenesis was altered and important phases of xylem development such as the radial-enlargement of tracheids were shortened. Similar extreme climatic events can impair the resilience of continental Mediterranean Scots pine forests subject to cold and drought stress. In addition, this impairment could affect

unexpected places as illustrated this case of winter-drought dieback trigger by a cold spell near the southernmost distribution limit of the species.

Author contribution statement

J.J. Camarero performed field sampling, led the writing and analyzed some data. G. Guada and E. Cervantes performed laboratory analyses. R. Sánchez-Salguero contributed to statistical analyses and made some figures. All authors discussed and contributed to the writing of the manuscript.

Acknowledgements

We thank several colleagues (Juan Manuel Gil, Víctor Pérez Fortea, Rodolfo Hernández, Miguel Ros and Araceli Ortiz) from the Mora de Rubielos laboratory (Aragón Government) for their sustained effort during field sampling. We also acknowledge the Spanish Agency of Meteorology (AEMET) for providing climate data. We thank J.J. Martín for taking the pictures under the confocal microscope. JJC acknowledges the support of the Excellence Network “Red de Ecología Terrestre para afrontar los retos del Cambio Global—ECOMETAS” (CGL2014-53840-REDT) of the Spanish Ministry of Economy.

References

- Abe, H., Nakai, T., Utsumi, Y., and Kagawa, A. 2003. Temporal water deficit and wood formation in *Cryptomeria japonica*. *Tree Physiology* 23: 859–863.
- Antonova, G.F., Cherkashin, V.P., Stasova, V.V. and Varaksina, T.N. 1995. Daily dynamics in xylem cell radial growth of Scots pine (*Pinus sylvestris* L.). *Trees-Structure and Function* 10: 24–30.
- Antonova, G.F. and Stasova, V.V. 1993. Effects of environmental factors on wood formation in Scots pine stems. *Trees-Structure and Function* 7: 214–219.
- Arend M, Fromm J. 2007. Seasonal change in the drought response of wood cell development in poplar. *Tree Physiology* 27: 985–992.
- Barnett JR. 1976. Rings of collapsed cells in *Pinus radiata* stemwood from lysimeter-grown trees subjected to drought. *New Zealand Journal of Forestry Science* 6: 461-465.
- Camarero, J. J., Guerrero-Campo, J. and Gutiérrez, E. 1998. Tree-ring structure and growth of *Pinus uncinata* Ram. and *Pinus sylvestris* L. in the Central Spanish Pyrenees. *Arctic and Alpine Research* 30: 1-10.
- Camarero JJ, Sancho-Benages S. 2006. Dendroecología, decaimiento del bosque y relaciones no lineales entre clima y crecimiento radial. In: JM Cuadrat, MA Saz, SM Vicente Serrano, S Lanjeri, M de Luis, JC González-Hidalgo, eds. *Clima, Sociedad y Medio Ambiente*. Zaragoza: Librería General, pp 1–9.
- Camarero JJ, Olano JM, Parras A. 2010. Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. *New Phytologist* 185: 471–480.
- Camarero, J.J., Gazol, A., Sancho-Benages, S. and Sanguesa-Barreda, G. 2015a. Know your limits? Climate extremes impact the range of Scots pine in unexpected places. *Annals of Botany* doi: 10.1093/aob/mcv124

- Camarero, J.J., Gazol, A., Sangüesa-Barreda, G., Oliva, J. and Vicente-Serrano, S.M. 2015b. To die or not to die: early-warning signals of dieback in response to a severe drought. *Journal of Ecology* 103: 44-57.
- Cinnirella, S.; Magnani, F.; Saracino, A. & Borghetti, M. 2002. Response of a mature *Pinus laricio* plantation to a three year restriction of water supply: structural and functional acclimation to drought. *Tree Physiology* 22: 21–30.
- Cook, E.R. and Krusic, P.J. 2005. Program Arstan, a tree-ring standardization program based on detrending and autoregressive time series modeling, with interactive graphics. Tree-Ring Laboratory, Lamont Doherty Earth Observatory, Columbia University, Palisades, NY.
- Cuny, H. E., Rathgeber, C. B. K., Frank, D., Fonti, P., and Fournier, M. 2014. Kinetics of tracheid development explain conifer tree-ring structure. *New Phytologist* 203: 1231–1241.
- Deslauriers, A., Morin, H. and Begin, Y. 2003. Cellular phenology of annual ring formation of *Abies balsamea* in the Quebec boreal forest (Canada). *Canadian Journal of Forest Research* 33: 190–200.
- Deslauriers, A. and Morin, H. 2005. Intra-annual tracheid production in balsam fir stems and the effect of meteorological variables. *Trees-Structure and Function* 19: 402– 408.
- Dobbertin, M. 2005. Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. *European Journal of Forest Research* 124: 319–333.
- Efron, B. and Tibshirani, R. 1993. *An Introduction to the Bootstrap*. Chapman & Hall, London.
- Eilmann, B., Dobbertin, M., and Rigling, A. 2013. Growth response of Scots pine with different crown transparency status to drought release. *Ann. For. Sci.* 70: 685–693.
- Eilmann, B., Zweifel, R., Buchmann, N., Graf Pannatier, E., and Rigling, A. 2011. Drought alters timing, quantity, and quality of wood formation in Scots pine. *J. Exp. Bot.* 62: 2763–2771.
- Fernández-De-Uña, L., Fonti, P., Aranda, I., Cañellas, I., and Gea-Izquierdo, G. 2013. Impact of drought on the intra-annual growth of *Pinus sylvestris* L. *Open For. Sci. J.* 6: 43–45

- Fritts, H.C. 2001. *Tree Rings and Climate*. Blackburn Press, Caldwell.
- Fukuda H. 1996. Xylogenesis: initiation, progression, and cell death. *Annual Review of Plant Physiology and Plant Molecular Biology* 47: 299–325.
- Gebauer R, Volařík D, Urban J, Børja I, Nagy NE, Eldhuset TD, Krokene P. 2015. Effects of prolonged drought on the anatomy of sun and shade needles in young Norway spruce trees. *Ecol. Evol.* 5: 4989–4998.
- Glerum C. 1970. Drought ring formation in conifers. *For. Sci.* 16: 246–248.
- Glerum C, Farrar JL. 1966. Frost ring formation in the stems of some coniferous species. *Can. J. Bot.* 44: 879–886.
- Glock, W.S. 1951. Cambial frost injuries and multiple growth layers at Lubbock, Texas. *Ecology* 32: 28–36.
- Gruber A, Strobl S, Veit B, Oberhuber W. 2010. Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. *Tree Physiol.* 30: 490–501.
- Guada G, Camarero JJ, Sánchez-Salguero R, Navarro-Cerrillo, RM. 2016. Limited growth recovery after drought-induced forest dieback in very defoliated trees of two pine species. *Frontiers in Plant Science* 7: 418. doi: 10.3389/fpls.2016.00418
- Hargreaves, G.H., and Samani, Z.A. 1982. Estimating potential evapotranspiration. *Journal of Irrigation and Drainage Engineering* 108: 225–230.
- Holmes, R.L. 1983. Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin* 43:69–78.
- Hsiao T.C., Acevedo E., Fereres E., Henderson D.W. 1976. Stress metabolism-water stress, growth, and osmotic adjustment. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 273:479–500
- Kullman L. 1989. Cold-induced dieback of montane spruce forests in the Swedish Scandes – a modern analogue of paleoenvironmental processes. *New Phytologist* 113: 377–389.
- Larcher, W. 1994. *Physiological Plant Ecology*. Springer, Berlin.

- Larson, P.R. 1994. The Vascular Cambium Development and Structure. Springer Verlag, Heidelberg-Berlin, Germany.
- Lazarus, B.E., Schaberg, P.G., DeHayes, D.H. and Hawley, G.J. 2004. Severe red spruce winter injury in 2003 creates unusual ecological event in the northeastern United States. Canadian Journal of Forest Research 34: 1784–1788.
- Leinonen I. 1996. A simulation model for the annual frost hardiness and freeze damage of Scots pine. Annals of Botany 78: 687–693.
- Matusick G, Ruthrof KX, Brouwers NC, Hardy GSJ. 2014. Topography influences the distribution of autumn frost damage on trees in a Mediterranean-type *Eucalyptus* forest. Trees-Structure and Function 28: 1449–1462.
- Mayr S, Schwienbacher F, Bauer H. 2003. Winter at the alpine timberline. Why does embolism occur in Norway spruce but not in Stone pine? Plant Physiology 131: 780–792.
- Müller, E. H. R., and H. R. Stierlin. 1990. Sanasilva tree crown photos with percentages of foliage loss. Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland.
- Oberhuber, W., Stumböck, M., Kofler, W. 1998. Climate tree–growth relationships of Scots pine stands (*Pinus sylvestris* L.) exposed to soil dryness. Trees-Structure and Function 13: 19–27.
- Pearce RS. 2001. Plant freezing and damage. Annals of Botany 87: 417–424.
- Peguero-Pina JJ, Alquézar-Alquézar JM, Mayr S, Cochard H, Gil-Peigrín E. 2011. Embolism induced by winter drought may be critical for the survival of *Pinus sylvestris* L. near its southern distribution limit. Annals of Forest Science 68: 565–574.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and R Core Team. (2015). nlme: Linear and Nonlinear Mixed Effects Models. Vienna: R package version 3.1-120, <http://CRAN.R-project.org/package=nlme>.

- R Development Core Team (2015) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
- Rathgeber, C.B.K., Longuetaud, F., Mothe, F., Cuny, H. and Le Moguédec, G. (2011). Phenology of wood formation, Data processing, analysis and visualisation using R (package CAVIAR). *Dendrochronologia* 29: 139–149.
- Rossi, S., Anfodillo, T. and Menardi, R. 2006a. Trephor: a new tool for sampling microcores from tree stems. *IAWA Journal* 27: 89–97.
- Rossi, S., Deslauriers, A. and Morin, H. 2003. Application of the Gompertz equation for the study of xylem cell development. *Dendrochronologia* 21: 33–39.
- Rossi, S., Deslauriers, A., Anfodillo, T., Morin, H., Saracino, A., Motta, R. and Borghetti, M. 2006b. Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. *New Phytol.* 170: 301–310.
- Sakai A, Larcher W. 1987. Frost survival of plants: responses and adaptation to freezing stress. Springer-Verlag, New York.
- Sánchez-Salguero R, Navarro-Cerrillo RM, Camarero JJ, Fernández-Cancio A. 2012. Selective drought-induced decline of pine species in southeastern Spain. *Climatic Change* 113: 767–785.
- Sánchez-Salguero, R., Camarero, J.J., Hevia, A., Madrigal-González, J., Linares, J.C., Ballesteros-Canovas, J.A., Sánchez-Miranda, A., Alfaro-Sánchez, R., Sangüesa-Barreda, S., Galván, J.D., Gutiérrez, E., Génova, M. and Rigling, A. 2015. What drives growth of Scots pine in continental Mediterranean climates: Drought, low temperatures or both? *Agric. For. Met.* 206: 151–162.
- Schweingruber, F.H. 2007. Wood Structure and Environment. Springer Series in Wood Science, Springer, 279 pp.

- Shortle, W. C., and Ostrofsky, A. (1983). Decay susceptibility of wood in defoliated fir trees related to changing physical, chemical, and biological properties. *Eur. J. For. Pathol.* 13: 1–11.
- Smith MD. 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. *Journal of Ecology* 99: 656–663.
- Soulé, P. T. and Knapp, P.A. 2007. Topoedaphic and morphological complexity of foliar damage and mortality within western juniper (*Juniperus occidentalis* var. *occidentalis*) woodlands following an extreme meteorological event. *J. Biogeogr.* 34: 1927–1937.
- Swidrak I, Gruber A, Kofler W, Oberhuber W. 2011. Effects of environmental conditions on onset of xylem growth in *Pinus sylvestris* under drought. *Tree Physiol.* 31: 483–498.
- Thabeet A, Vennetier M, Gadbin-Henry C, Denelle N, Roux M, Caraglio Y, Vila B. 2009. Response of *Pinus sylvestris* L. to recent climatic events in the French Mediterranean region. *Trees-Structure and Function* 23: 843–853.
- Torelli, N., Shortle, W. C., Cufar, K., Ferlin, F. and Smith, K. T. 1999. Detecting changes in tree health and productivity of silver fir in Slovenia. *Eur. J. For. Pathol.* 29: 189–197.
- Voltas J, Camarero JJ, Carulla D, Aguilera M, Ortiz A, Ferrio JP. 2013. A retrospective, dual-isotope approach reveals individual predispositions to winter-drought induced tree dieback in the southernmost distribution limit of Scots pine. *Plant, Cell Env.* 36: 1435–1448.
- Wodzicki, T.J. 1971. Mechanism of xylem differentiation in *Pinus sylvestris* L. *J. Exp. Bot.* 22: 670–687.
- Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. and Smith, G.M. 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

Tables

Table 1. Characteristics of the study Scots pine trees measured in 2005 and growth data (number of tracheids; BAI, basal area increment). Different letters indicate significant differences ($P < 0.05$) between tree types within each study site (Mann-Whitney U tests). Sites are abbreviated as in Table 1. Values are means \pm SE.

Variable	Villarroya de los Pinares		Torrijas	
	Non-declining trees	Declining trees	Non-declining trees	Declining trees
No. trees	10	10	10	10
Dbh (cm)	32.0 \pm 1.7	32.8 \pm 1.1	34.4 \pm 2.1	31.9 \pm 1.2
Height (m)	13.4 \pm 0.3	12.7 \pm 0.3	17.0 \pm 0.5	15.5 \pm 0.4
Age (years)	125 \pm 15	122 \pm 20	115 \pm 17	117 \pm 15
Horizontal crown diameter (m)	4.8 \pm 0.5	5.7 \pm 0.4	6.9 \pm 0.4	6.1 \pm 0.4
Crown cover in 2005 (%)	98 \pm 2b	35 \pm 3a	97 \pm 1b	44 \pm 3a
No tracheids in 2002 tree ring	33 \pm 3b	21 \pm 4a	47 \pm 8b	26 \pm 4a
No tracheids in 2003 tree ring	28 \pm 3b	16 \pm 2a	36 \pm 7b	16 \pm 3a
No tracheids in 2004 tree ring	31 \pm 3b	11 \pm 2a	40 \pm 5b	15 \pm 3a
No tracheids in 2005 tree ring	25 \pm 3b	8 \pm 2a	32 \pm 4b	10 \pm 2a
Relative No. 2005 tracheids (%)	98 \pm 9b	63 \pm 11a	93 \pm 15b	53 \pm 12a
BAI 1965-1997 period (cm ²)	4.2 \pm 0.2a	5.7 \pm 0.2b	0.9 \pm 0.2	1.0 \pm 0.1
BAI 1998-2001 period (cm ²)	4.5 \pm 0.4	5.0 \pm 0.4	2.7 \pm 0.6	2.0 \pm 0.4
BAI 2002-2005 period (cm ²)	4.8 \pm 0.4b	1.4 \pm 0.3a	2.9 \pm 0.6b	0.9 \pm 0.2a

Table 2. Characteristics of the non-declining ($n = 10$) and declining ($n = 10$) trees monitored in the Villarroya de los Pinares site (VP) to describe aboveground biomass components. Values are means \pm SD. Different letters indicate significant differences ($P < 0.05$) between tree types within each study site (Mann-Whitney U tests).

Variable	Non-declining trees	Declining trees
Aboveground biomass (g m^{-2})	899 \pm 80b	565 \pm 44a
Needle mass (g m^{-2})	505 \pm 60b	310 \pm 39a
Bark mass (g m^{-2})	181 \pm 29b	119 \pm 10a
Shoot mass (g m^{-2})	142 \pm 15b	101 \pm 10a
Male cones mass (g m^{-2})	51 \pm 8b	14 \pm 2a
Frequency of dead apical buds (%)	2.1 \pm 0.9a	77.4 \pm 3.4b
Frequency of shoots with male cones (%)	52.1 \pm 12.0b	1.5 \pm 1.5a
Shoot length (mm)	26.7 \pm 2.1b	4.3 \pm 0.3a
Needle length (mm)	32.8 \pm 1.3a	47.1 \pm 1.8b

Table 3. Coefficients (a , b , c) and statistics (R^2 , P , SE –standard error) of the Gompertz ($y = a e^{-(e^{b-cx})}$) functions fitted to the production of mature tracheids by declining and non-declining trees in the two study sites (Villarroya de los Pinares, Torrijas). Coefficients and statistics are given as means \pm standard error (SE). All fits were highly significant ($P < 0.001$). The following parameters are presented for the Gompertz function: t_p , date of the inflection point of the function; r , absolute mean rate; and t_d , day at which most annual growth is attained. Data refer to the 2005 study year. Different letters indicate significant differences ($P < 0.05$) between tree types within each study site (Mann-Whitney U tests).

Parameter or statistic	Villarroya de los Pinares		Torrijas	
	Non-declining trees	Declining trees	Non-declining trees	Declining trees
a (No. tracheids)	38 \pm 5b	9 \pm 2a	23 \pm 3b	10 \pm 2a
b	1.34 \pm 0.07	1.35 \pm 0.08	1.95 \pm 0.20	1.79 \pm 0.12
c	0.02 \pm 0.01	0.03 \pm 0.01	0.05 \pm 0.02	0.04 \pm 0.01
R^2 (%)	99.4 \pm 2.3	94.2 \pm 1.5	99.4 \pm 2.0	95.1 \pm 1.7
SE	1.1 \pm 0.4	0.8 \pm 0.3	0.7 \pm 0.2	0.8 \pm 0.2
r (tracheids day ⁻¹)	0.19 \pm 0.04b	0.06 \pm 0.01a	0.26 \pm 0.04b	0.11 \pm 0.02a
t_p (day of the year) [#]	163 \pm 4b	151 \pm 3a	143 \pm 3b	132 \pm 2a
t_d (day of the year) [§]	295 \pm 9b	259 \pm 8a	188 \pm 5	186 \pm 4

[#]Days of the year are equivalent to the following Julian days: 163, 12 June; 151, 31 May; 143, 23 May; 132, 12 May.

[§]Days of the year are equivalent to the following Julian days: 295, 22 October; 259, 16 September; 186, 5 July; 188, 5 July.

Table 4. Statistical tests obtained by comparing the estimated onset and cessation dates and the duration of the main phases of tracheid differentiation (radial enlargement, cell-wall thickening and lignification, xylem formation) for the Torrijas and Villarroya de los Pinares study sites and considering declining (D) and non-declining (N) Scots pines. The last column shows the achieved significance level (ASL), which is analogously interpreted to a *P* significance level since the smaller the value of ASL, the stronger the evidence against the null hypothesis (no difference between vigour classes) Significant ($P < 0.05$) ASL values appear in bold characters.

Site	Xylogenesis phase	Xylogenesis phase	ASL
Torrijas	Onset	Radial enlargement	0.063
		Cell-wall thickening	0.054
	Cessation	Radial enlargement	0.138
		Cell-wall thickening	0.062
	Duration	Radial enlargement	0.272
		Cell-wall thickening	0.040
Xylem formation		0.046	
Villarroya de los Pinares	Onset	Radial enlargement	0.024
		Cell-wall thickening	0.020
	Cessation	Radial enlargement	0.013
		Cell-wall thickening	0.025
	Duration	Radial enlargement	0.001
		Cell-wall thickening	0.042
Xylem formation		0.038	

Table 5. Parameters and statistics of linear mixed-effects models of xylogenesis data (number of cambial cells, number of radially enlarging and wall thickening tracheids) and rate of production of mature tracheids in declining and non-declining Scots pine trees from the two study sites. The adjusted R² (R²adj) measures the proportion of variance of the variable explained by the model adjusting for the number of terms of the model. All models were evaluated for 7 and 15-day long intervals. Bold values are significant estimates ($P < 0.05$).

Site (code)	Variables	No. cambial cells		No. radially-enlarging tracheids		No. wall-thickening and lignifying tracheids		Production rate of mature tracheids	
		7 days	15 days	7 days	15 days	7 days	15 days	7 days	15 days
Villarroya de los Pinares (VP)	Tree	0.01		0.02		0.03		0.07	
	Vigour class	0.03		0.50		0.55		2.03	
	Time	0.02		0.05		0.04		0.07	
	Maximum Temperature	0.01	0.05	0.01	0.02	0.02	0.02	0.05	0.10
	Minimum Temperature	0.02	0.06	0.02	0.03	0.03	0.09	0.05	0.09
	Relative Humidity	-0.02	-0.02	0.01	0.01	-0.01	0.01	0.04	0.01
	Day length	0.02	0.06	0.16	0.11	0.04	0.05	0.10	0.19
	Precipitation	0.02	0.04	0.01	0.04	-0.06	-0.03	0.05	0.06
	Water balance	0.01	0.05	0.02	0.05	-0.07	-0.04	0.04	0.05
	R ² adj (%)	18.25	19.80	56.96	54.93	54.56	41.02	80.54	78.05
Torrijas (TO)	Tree	0.01		0.03		0.03		0.08	
	Vigour class	0.03		0.16		0.29		1.14	
	Time	0.02		0.02		0.06		0.06	
	Maximum Temperature	0.02	0.04	0.07	0.05	0.02	0.25	0.05	0.08
	Minimum Temperature	0.04	0.05	0.08	0.05	0.01	0.30	0.04	0.07
	Relative Humidity	0.01	0.03	0.01	0.01	-0.01	-0.05	0.01	0.05
	Day length	0.03	0.05	0.10	0.08	0.07	0.01	0.08	0.07
	Precipitation	0.04	0.05	0.09	0.12	-0.01	-0.04	0.04	0.06
	Water balance	0.05	0.06	0.08	0.11	-0.02	-0.05	0.03	0.06
	R ² adj (%)	20.14	19.42	62.79	63.48	50.00	58.10	88.70	88.89

Figures

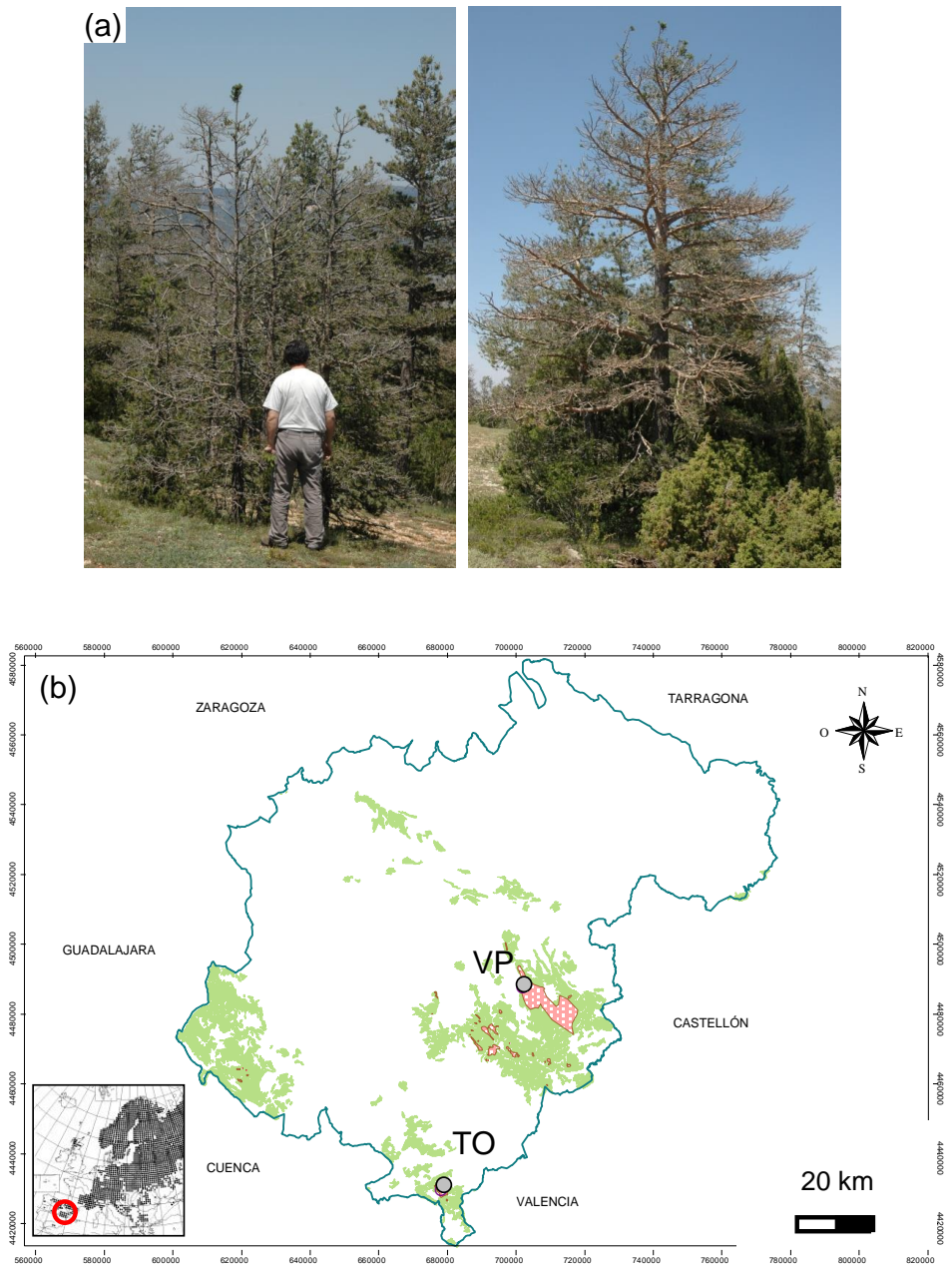


Figure 1. Views of Scot pine trees affected by the winter drought-induced dieback (a) in the study area located in Teruel province, eastern Spain (b). The map show areas affected by winter drought-induced dieback (red patches) as compared with the Scots pine distribution area in the Teruel province (green patches). The distribution area of Scots pine in Europe is shown in the lowermost plot, left inset. The two dots correspond to the two sampled sites: Villarroya de los Pinares (VP, Gúdar range) and Torrijas (TO, Javalambre range). Note that the study area is located near the southernmost limit of the continuous Scots pine distribution area in Europe.

Comentario [E1]: Debe ser Scots

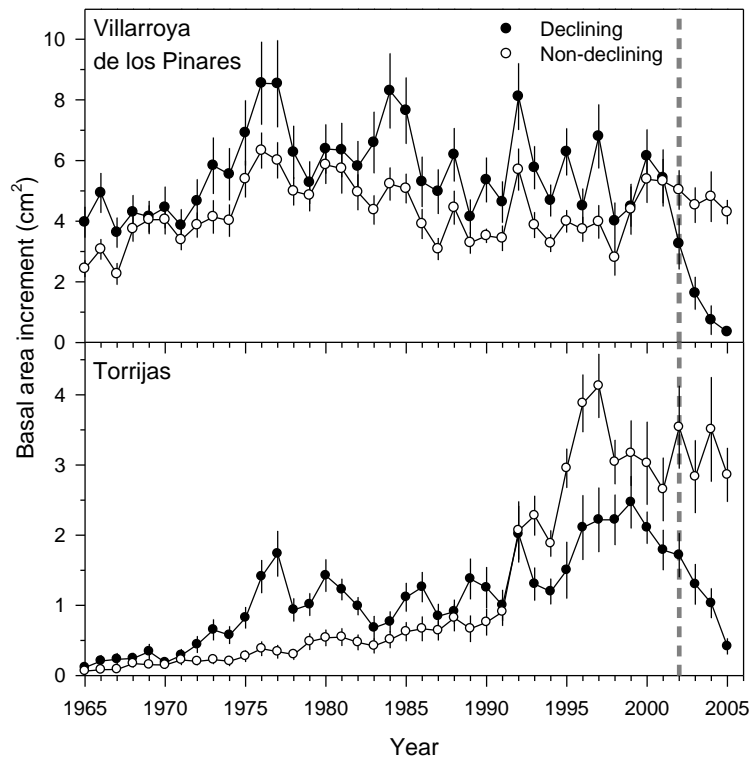


Figure 2. Trends in basal area increment (BAI) observed in declining (filled symbols) and non-declining (empty symbols) trees in the Villarroya de los Pinares and Torrijas study sites. Values are means \pm SE. The vertical dashed line highlights the 2002 year when BAI data started to diverge between the two classes of trees.

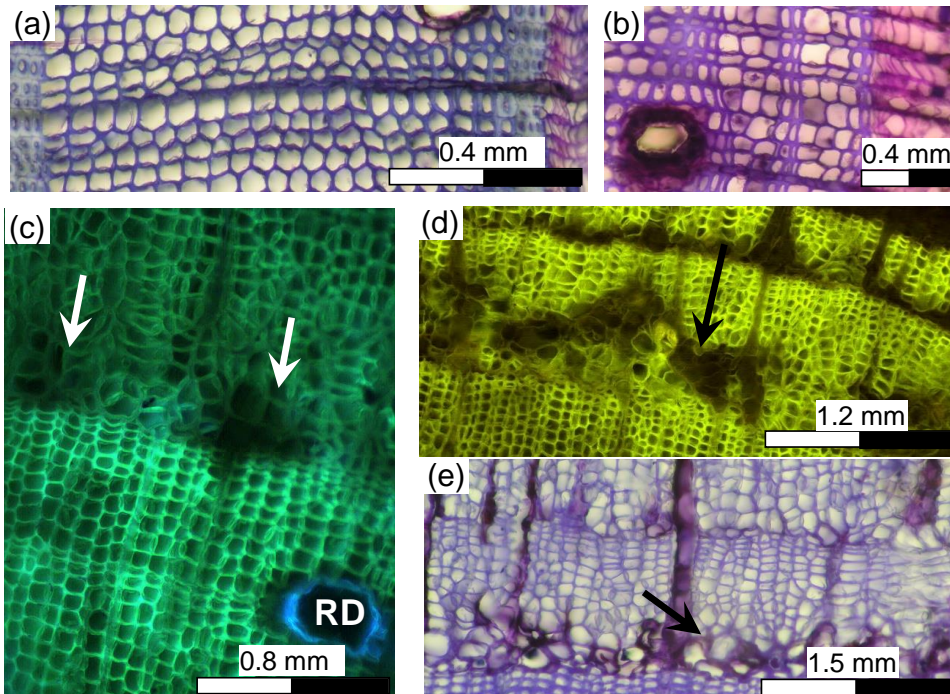


Figure 3. Cross-sections of tree rings formed during 2005 (the images correspond to October samples) by a non-declining (a) and a declining (b) Scots pine trees and views of abnormal xylem anatomical features (c, d, e). The lowermost images (c, d, e) show areas with collapsed earlywood tracheids (arrows) observed in the 2002 tree ring of declining trees. In (c) a resin duct is indicated (RD). Images (c) and (d) were obtained using confocal microscopy. All images correspond to trees located in the Villarroya de los Pinares (VP) study site.

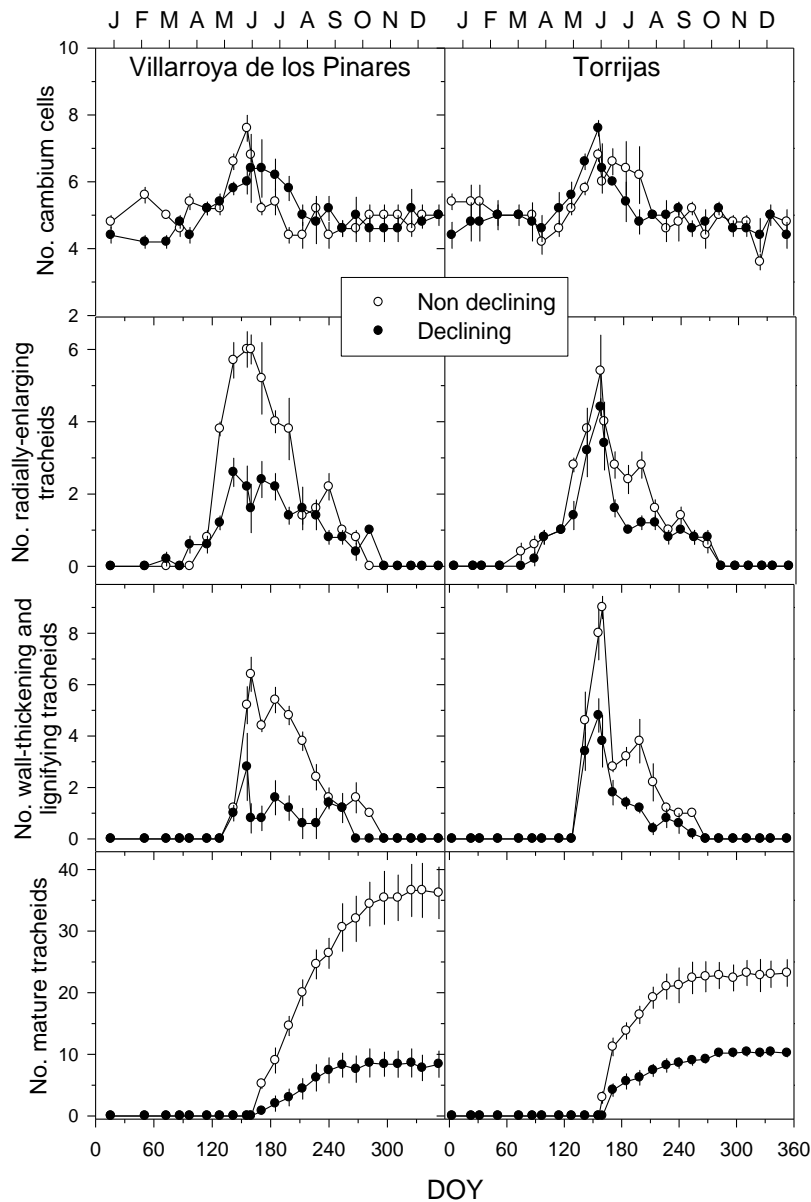


Figure 4. Numbers of cambium cells and tracheids along different xylogenesis phases (radial enlargement, cell-wall thickening and lignification, maturation) produced by non-declining (empty symbols) and declining (filled symbols) trees in the Villarroya de los Pinares (VP) and Torrijas (TO) study sites. Values are means \pm SE. The lowermost axis showed the day of the year (DOY) during 2005.

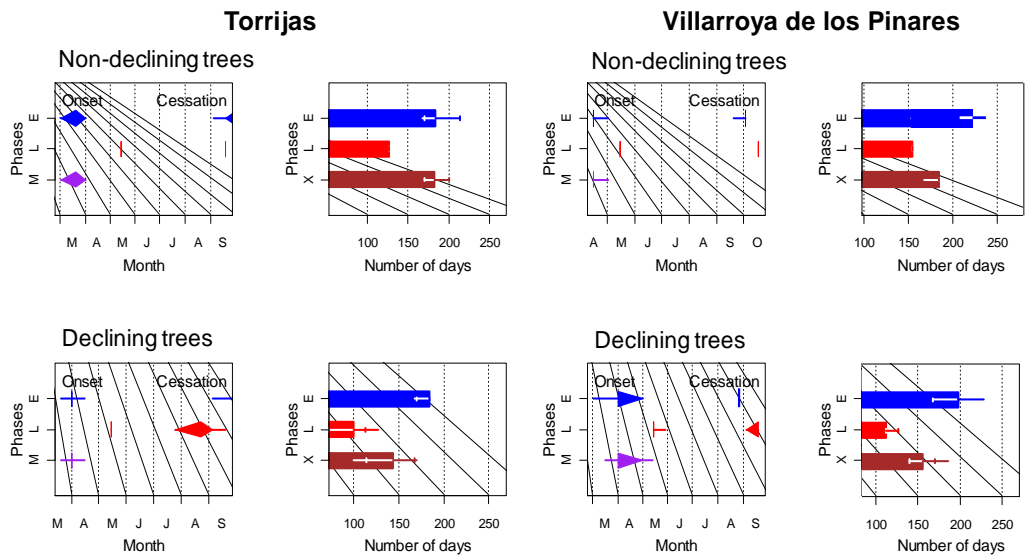


Figure 5. Estimated onset and cessation dates and durations (mean \pm SD) of the main phases of xylogenesis (E, radial enlargement; L, cell-wall thickening and lignification; M, mature tracheids; X, xylem formation) in non-declining and declining Scots pine trees sampled in the Torrijas and Villarroya de los Pinares study sites. The onset and cessation dates of selected xylogenesis phases are represented by diamond-crossed-by-a-line marks whose left (right) end of the line represents the minimum (maximum), the left (right) end of the diamond the first (third) quartile and the middle of the diamond corresponds to the median.

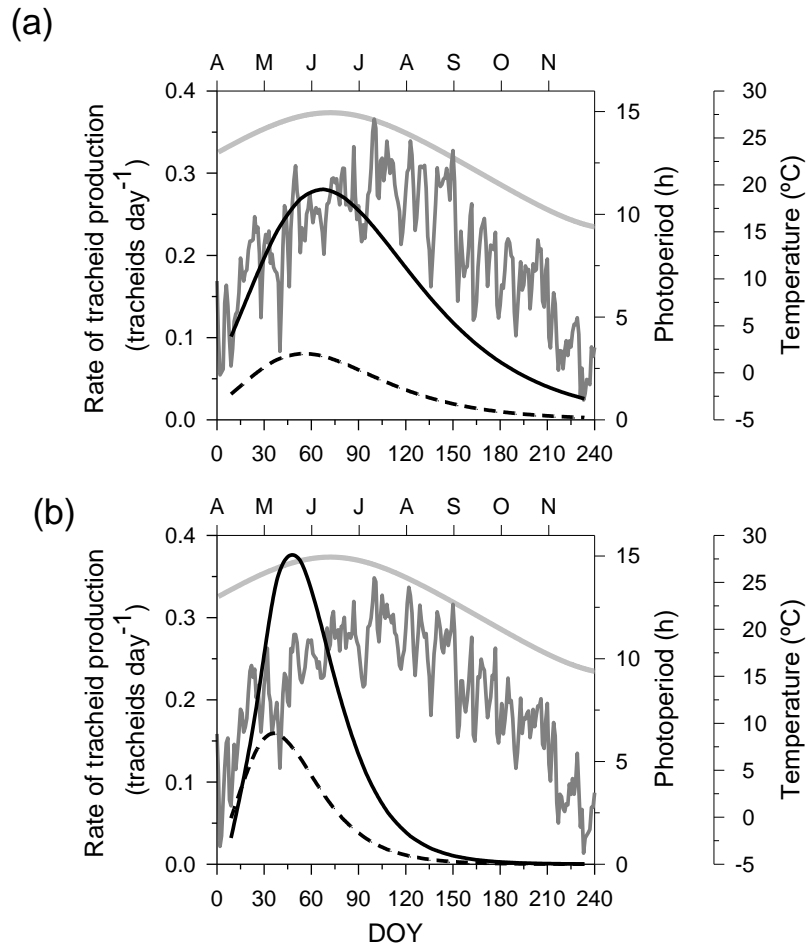


Figure 6. Modeled mean production rates of mature tracheids for non-declining (continuous black lines) and declining (dashed black lines) trees in the (a) Villarroya de los Pinares and (b) Torrijas study sites as related to climatic variables (photoperiod, smooth grey line; mean temperature, grey line). Rates were obtained after fitting Gompertz functions to tracheid data as a function of time (DOY, day of the year). Note that fitted rates are mean values for each tree class and standard errors are not presented for visual clarity.

Supporting Information

Figure S1. Climatic diagrams of two meteorological stations located near the two study sites (site VP, station Villarroya de los Pinares, 40° 31' 48'' N, 0° 40' 10'' W, 1337 m a.s.l.; period 1990-2004; site TO, station Torrijas-Los Cerezos, 40° 01' 20'' N, 0° 57' 27'' W, 1356 m a.s.l.; period 1953-2004).

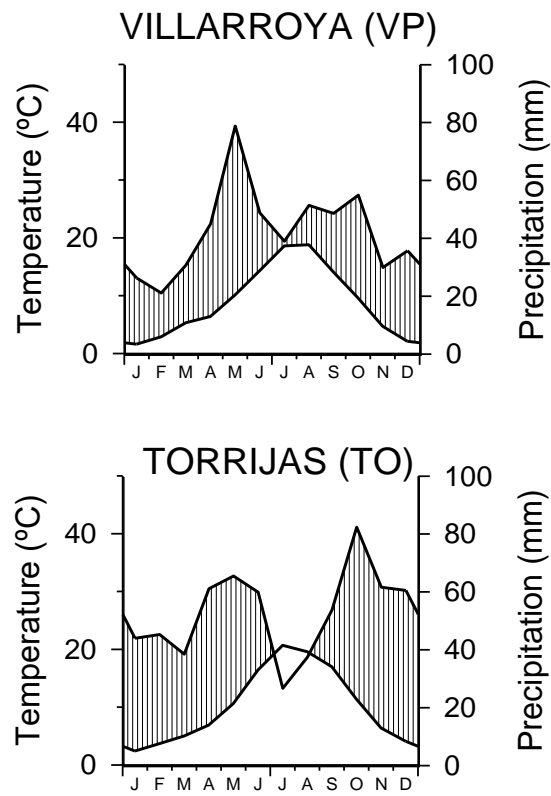


Figure S2. Climatic conditions during the study year (2005) considering data from a northern- (N) and a southern-oriented (S) data loggers placed in tree stems and compared with data from nearby meteorological stations. The smoothed lines show day length, whereas the bars indicate precipitation (P). In the lowermost plots of each site, relative air humidity (RH) is plotted with lines.

