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Research paper

Impact of successive spring frosts on leaf phenology and radial growth in three deciduous tree species with contrasting climate requirements in central Spain

Álvaro Rubio-Cuadrado[1,5], J. Julio Camarero[2], Jesús Rodríguez-Calcerrada[1], Ramón Perea[1], Cristina Gómez[3], Fernando Montes[4] and Luis Gil[1]

[1]Departamento de Sistemas y Recursos Naturales, Escuela Técnica Superior de Ingeniería de Montes, Forestal y del Medio Natural, Universidad Politécnica de Madrid, c/ José Antonio Novais, 10, Madrid 28040, Spain; [2]Instituto Pirenaico de Ecología (IPE-CSIC), Avda Montañana 1005, Zaragoza 50080, Spain; [3]iuFOR-EiFAB, Campus Duques de Soria, Universidad de Valladolid, Soria 42004, Spain; [4]INIA, Forest Research Centre, Department of Silviculture and Forest Management, Crta La Coruña km 7.5, Madrid 28040, Spain; [5]Corresponding author (alvaro.rubio.cuadrado@upm.es, alvarorubiocuadrado@gmail.com)

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Rear-edge tree populations forming the equatorward limit of distribution of temperate species are assumed to be more adapted to climate variability than central (core) populations. However, climate is expected to become more variable and the frequency of climate extremes is forecasted to increase. Climatic extreme events such as heat waves, dry spells and spring frosts could become more frequent, and negatively impact and jeopardize rear-edge stands. To evaluate these ideas, we analyzed the growth response of trees to successive spring frosts in a mixed forest, where two temperate deciduous species, *Fagus sylvatica* L. (European beech) and *Quercus petraea* (Matt.) Liebl. (sessile oak), both at their southernmost edge, coexist with the Mediterranean *Quercus pyrenaica* Willd. (Pyrenean oak). Growth reductions in spring-frost years ranked across species as *F. sylvatica* > *Q. petraea* > *Q. pyrenaica*. Leaf flushing occurred earlier in *F. sylvatica* and later in *Q. pyrenaica*, suggesting that leaf phenology was a strong determinant of spring frost damage and stem growth reduction. The frost impact depended on prior climate conditions, since warmer days prior to frost occurrence predisposed to frost damage. Autumn Normalized Difference Vegetation Index data showed delayed leaf senescence in spring-frost years and subsequent years as compared with pre-frost years. In the studied forest, the negative impact of spring frosts on *Q. petraea* and especially on *F. sylvatica* growth, was considerably higher than the impacts due to drought. The succession of four spring frosts in the last two decades determined a trend of decreasing resistance of radial growth to frosts in *F. sylvatica*. The increased frequency of spring frosts might prevent the expansion and persistence of *F. sylvatica* in this rear-edge Mediterranean population.

***Keywords*:** late frost, leaf unfolding, legacy, Mediterranean forest, resilience, temperate forest.

Introduction

Climate change may increase the frequency and severity of extreme climate events such as droughts and frosts, amplifying the consequences of climate change and challenging the ability of tree species to adapt to the new conditions (Stott 2016). In the case of spring frosts, i.e., below-zero temperatures occurring after the beginning of the vegetative period, Ma et al. (2019) and Zohner et al. (2020) have shown that the frequency of their occurrence has changed differently across geographic areas and ecosystems, having generally increased since 1959 in Europe. These results reconcile apparently contradictory predictions of decreasing frost-risk in some studies (Menzel et al. 2003, Scheifinger et al. 2003, Dai et al. 2013, Morin and Chuine 2014, Hänninen 2016) and increasing risk in others

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(Cannell 1985, Kramer 1994, Gu et al. 2008, Inouye 2008, Augspurger 2013, Vitasse et al. 2014, Hänninen 2016, Abbas et al. 2017, Bigler and Bugmann 2018).

Spring frosts are critical events that could cause widespread damage to forest ecosystems, such as abrupt reductions in productivity, demographic changes and shifts in species composition (Augspurger 2009, 2011), especially if these become commonplace due to climate change (Körner et al. 2016). At the tree level, a spring frost may damage the leaves, affecting their morphology, physiology and chemical composition (Klosson and Krause 1981*a*, 1981*b*, Sakai and Larcher 1987). The roots and live stem tissues are insulated by the soil and the outer bark, respectively, and are less likely to be damaged. Still, shallow fine roots, and young and large xylem conduits can be affected even by light frosts (Neuner et al. 2010, Ambroise et al. 2020). When the leaves are completely scorched by a spring frost, trees have to rebuild new foliage by mobilizing several-years-old reserves of non-structural carbohydrates and nutrients (D'Andrea et al. 2019). The time to rebuild a new leaf cohort depends in part on the capacity to remobilize the stored carbohydrates and nutrients, and, if the xylem was embolized by freeze–thaw cycles, it also depends on the capacity to produce new xylem conduits. The second cohort of leaves may be delayed by up to 1 month or more from the first cohort (Nolè et al. 2018, D'Andrea et al. 2019), resulting in a loss of photosynthesis. Once developed, this cohort of leaves may be less productive due to lower leaf nutrient concentrations, leaf size and/or total number of leaves (Awaya et al. 2009). Thus, frost-damaged trees experience a reduction in carbon uptake, which may lead to a reduction of up to 90% or more in radial growth (Dittmar et al. 2006). D'Andrea et al. (2021) showed that a spring frost can reduce the carbon stores of trees, especially starch concentrations, but they would return to a normal level during the following year. According to Körner (2015), in general, the rates of photosynthesis are controlled by the growth of tissues and, therefore, the repercussion of late frosts on growth would mostly be due to the reduction of the vegetative period caused by the inability of cells to duplicate at low temperatures and by the hormonal signals induced. In any case, a single frost, but more likely a succession of frosts, could permanently damage the trees and kill them several years later, similar to the drought stress legacy effects that give rise to massive mortality episodes (Anderegg et al. 2015, Rodríguez-Calcerrada et al. 2017, Kannenberg et al. 2020).

In addition to the direct impact of the spring frosts damaging or killing the leaves, these events may have an indirect effect on trees by modifying the leaf phenology throughout the year of frost. In this sense, previous studies linked autumn phenology with the preceding spring development, so that delays in leaf flushing would be compensated for by delays in leaf senescence (Fu et al. 2014, Keenan and Richardson 2015, Liu et al. 2016, Signarbieux et al. 2017, Zohner et al. 2018). In contrast, the results obtained in other studies do not show such a relationship (Awaya et al. 2009, Bascietto et al. 2018).

The leaf emergence in deciduous tree species is mainly coupled with minimum temperatures (Lenz et al. 2013, Körner et al. 2016, Vitasse et al. 2018), but other factors, such as photoperiod, may have a strong influence on the phenology of some species, such as *Fagus sylvatica* L. (Vitasse et al. 2009). Although deciduous trees exhibit similar safety margins to avoid leaf freezing damage by spring frost (i.e., similar difference between the minimum temperature experienced and the freezing resistance of the species; Lenz et al. 2013), the variability in frost resistance between species results in different leaf flushing times (Körner et al. 2016). Therefore, the leaves of early flushing species are more likely to be damaged by a spring frost than those of later flushing coexisting species (Augspurger 2009). The study of leaf phenology can be of great importance to determine the direct impact of spring frosts and their indirect effects. Since phenological information based on direct observation is not frequently available, spectral indices like the Normalized Difference Vegetation Index (NDVI) are frequently used. The NDVI has proved effective to quantify changes in aboveground phenology after late frosts, and to estimate the canopy area affected and the loss in productivity, as well as the duration of the freezing stress effects (Bascietto et al. 2018, Greco et al. 2018, Nolè et al. 2018).

Rear-edge tree populations face harsh climate conditions and will be particularly affected by climate warming and the increase in extreme climate events (Hampe and Petit 2005). This is the case of some populations of temperate species in Mediterranean mountains (Rubio-Cuadrado et al. 2018*a*, Gazol et al. 2019), characterized by warm and dry summer conditions (Giorgi and Lionello 2008). In this work, we studied a mixed forest in central Spain, which is the southernmost distribution limit of *F. sylvatica* and *Quercus petraea* (Matt.) Liebl. (Pardo et al. 2004). Here, both temperate species co-occur with the Mediterranean *Quercus pyrenaica* Willd. These three broadleaved deciduous species show different leaf phenology, with *F. sylvatica* sprouting earlier than both *Quercus* species. Our targeted forest has been monitored since 1994. Since then, there have been conspicuous leaf damages due to spring frosts in at least one of the three dominant species in 1995, 2010, 2013 and 2017. Our objectives in this work were: (i) to evaluate the difference in growth among *F. sylvatica Q. petraea* and *Q. pyrenaica* during the years with spring frost; (ii) to study the post-frost growth resilience of the three tree species; and (iii) to analyze NDVI 5-year series including a central episode of spring frost, relating them to frost-impact and post-frost effects. We hypothesized that: (i) the radial growth would be more affected in *F. sylvatica* than in the two *Quercus* species during the spring frost years due to the earlier leaf flushing of *F. sylvatica*; (ii) radial growth would recover more slowly in *F. sylvatica* than in both *Quercus* due to frost legacies; and (iii) stand primary

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productivity would be reduced during spring frost years and subsequent years, whereas leaf flushing and senescence would be delayed in spring frost years.

Materials and methods

Study area

The study site, 'El Hayedo de Montejo', is a mixed forest of 125 ha located at the 'Sistema Central' mountain range, central Spain (41° 07′ N, 3° 30′ W), between 1250 and 1500 m above sea level (a.s.l.) The site aspect is east and the dominant species are the Mediterranean *Q. pyrenaica* and the temperate *F. sylvatica* and *Q. petraea*, with relative basal areas of 50, 27 and 23% and mean diameters at breast height of 24.1, 16.5 and 19.9 cm, respectively. *Fagus sylvatica* and *Q. petraea*, as well as most individuals of *Q. pyrenaica*, originated from natural seedling in the studied forest, which contrasts with the coppice stands of *Q. pyrenaica* so characteristic in Central Spain (Valbuena-Carabaña et al. 2008). During centuries of firewood harvesting and cattle grazing, the forest had an open woodland structure of dispersed old and large trees, and scarce tree recruitment (Pardo and Gil 2005). However, in 1961 cattle grazing was forbidden, and the last logging took place in 1962 (López Santalla et al. 2003, Gil et al. 2009). These changes in land use have favored an increase in the number of trees during the last decades.

The climate is continental Mediterranean, with 900 mm of mean annual rainfall and a prolonged dry period during July and August. The mean annual temperature is 9.5 °C. Temperatures below 0 °C occur from November to February, but spring frosts are also common. The soil has been classified as humic cambisol and the horizon A reaches 50 cm of depth on average, enabling considerable water storage during dry periods (Pardo et al. 1997).

Frost events

Climate data were obtained from a meteorological station set up in the study forest in 1994, at 1406 m a.s.l., and managed by the Natural Systems and Forest History research group of 'Universidad Politécnica de Madrid', Spain. Missing data of the minimum temperatures were estimated by a linear regression model of data from this station and those from 'Pantano El Vado' local station (41° 0′ 13″ N, 3° 18′ 7″ W, 910 m a.s.l.; AEMET, Spanish Meteorological Agency), located ca 21-km away from the forest $\left(R^2 = 0.76\right)$:

$$\mathrm{Tmin}_{\text{El Hayedo de Montejo}} = 0.864\ \mathrm{Tmin}_{\text{Pantano El Vado}} + 0.1547 \tag{1}$$

Since 1994, there have been four spring frosts damaging the leaves of one or more of the tree species coexisting in the studied forest (Figure 1). The spring frosts occurred on 23–26 April 1995, when temperatures reached a negative peak of −2.4 °C; 3–6 May 2010, when temperatures reached −2.0 °C; 27–28 April 2013, when temperatures reached −3.8 °C; and 27–28 April 2017, when temperatures reached −4.6 °C. Furthermore, in these 4 years there were also secondary frosts occurring after the main damaging frost, which might have increased the impact produced. Secondary frosts occurred on: 12–13 May 1995, when temperatures reached −1.25 °C; 14 May 2010, when temperatures reached −1.85 °C; 17–18 May 2013, when temperatures reached −0.16 °C; and 1 May 2017, when temperatures reached −1.63 °C.

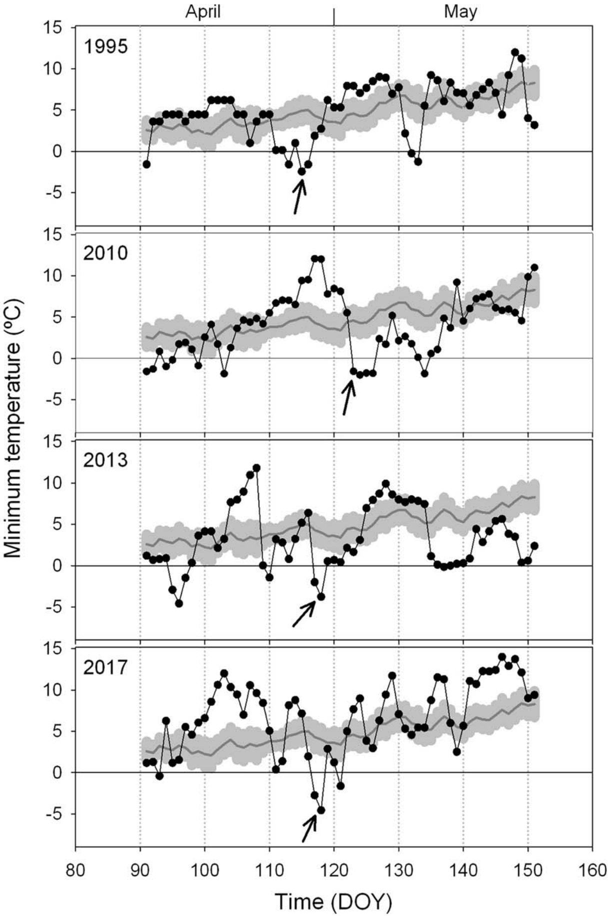

Figure 1. Daily minimum air temperatures from April to May in years with frost damage (1995, 2010, 2013 and 2017) compared with an average year for the 1994–2018 period (with dark gray lines for the mean and light gray area for the 95% confidence intervals). The arrows point to days in which the frost damage was observed.

Leaf phenology

In order to relate the time of bud opening and leaf emergence with the spring frost damage, we retrieved a phenological inventory performed in 10 *F. sylvatica* adult trees, 8 *Q. petraea*

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and 8 *Q. pyrenaica* adult trees (Millerón et al. 2012). In this inventory three twigs from branches oriented to the south and three twigs from branches oriented to the north were monitored on each tree once a week during the whole vegetative period to examine leaf phenology from 2006 to 2011. Sampled twigs were located in the lower part of the live crown (<3-m height). One-year-old twigs were selected, unless they had fewer than five buds, in which case longer (older) twigs were selected. For each twig, the number of still-dormant buds and the foliar phenophase were annotated following Millerón et al. (2012). We recorded leaf flushing time (in Julian day of the year [DOY]) and then we estimated its temporal range for each species, with the median and the 10th, 25th, 75th and 90th percentiles, since neither the buds of a given tree nor the individuals of a given species sprouted simultaneously.

Sampling and dendrochronological analyses

Between 20 and 22 dominant or codominant trees distributed throughout the forest were selected for each species. In each tree two wood cores were extracted at 1.3 m (always perpendicular to the maximum slope), using a Pressler increment borer, and the diameter at breast height (DBH) of each tree was measured at 1.3 m with a caliper during autumn of 2018. Cores were mounted on wooden supports and carefully sanded until tree rings were clearly visible. After a visual cross-dating, tree-ring widths were measured to the nearest 0.01 mm using the semi-automatic LINTAB measuring device with the TSAP-Win software (RINNTECH, Heidelberg, Germany). Then, visual cross-dating was further verified with the COFECHA program (Holmes 1997). Growth data are available in Rubio-Cuadrado et al. (2020*b*). Altitudes for sampled trees were retrieved from a Digital Terrain Model of 25-m spatial resolution (PNOA, Instituto Geográfico Nacional, Spain).

We calculated the mean tree-ring width series for each individual tree. These series were subsequently transformed into basal area increments (BAI), as this variable is better than tree-ring width for capturing growth trends and accounting for the increase in size and age of stems (Biondi and Qeadan 2008). The BAI was calculated as follows:

$$\mathrm{BAI} = \pi\left(r_t^2 - r_{t-1}^2\right) \qquad (2)$$

where r_t and r_{t-1} are the stem radii at the end and the beginning of a given annual ring.

When cores did not contain the pith, the missing core length was estimated to calculate the BAI by subtracting the core length to the tree radius measured in the field without bark. Bark thickness was estimated based on tree diameter using equations fitted to the data from the Second Spanish National Forest Inventory (DGCONA 1998). Functions used to estimate the bark thickness of *F. sylvatica* (C_{Fs}), *Q. petraea* (C_{Qpe}) and *Q. pyrenaica* (C_{Qpy}) are the following:

$$C_{Fs} = -0.00001\ \mathrm{DBH}^2 + 0.0284\ \mathrm{DBH} + 0.3545 \qquad (3)$$

$$C_{Qpe} = 1.4407 \cdot \mathrm{DBH}^{0.4071} \qquad (4)$$

$$C_{Qpy} = 7.4422\ \ln(\mathrm{DBH}) - 23.845 \qquad (5)$$

The final species chronologies were built by averaging annual BAIs across all trees of the same species. The statistical quality of each chronology or mean site series was checked via expressed population signal (EPS; Speer 2012), which measures replication and coherence as compared with a perfectly replicated chronology. A threshold value of EPS >0.85 was considered reliable to represent the mean BAI series of each tree species. The description of dendrochronological variables of cored trees is shown in Table S1 available as Supplementary data at *Tree Physiology* Online.

Impact of spring frosts on tree growth

Individual tree growth reduction due to spring frost events was characterized via the tree-ring series, calculating resistance (*Rt*) and resilience (*Rs*) indices (Lloret et al. 2011) as:

$$Rt = \mathrm{BAI}_i/\mathrm{BAI}_{i-6} \qquad (6)$$

$$Rs = \mathrm{BAI}_i + 1/\mathrm{BAI}_{i-6} \qquad (7)$$

where BAI_i is the BAI observed in the frost year, BAI_{i-6} is the mean BAI calculated over the 6 years preceding the frost year, and BAI_{i+1} is the observed BAI in the year that follows the frost year. *Rt* and *Rs* were calculated using the *pointRes* package in R (van der Maaten-Theunissen et al. 2015). Although these indices are typically calculated over periods of 3–4 years after the stress event when studying drought effects (Gazol et al. 2017, Rubio-Cuadrado et al. 2018*a*), we considered just 1 year after the spring frosts because it is apparently the only year in which growth is affected, in addition to the year of the spring frost itself (see Results), and because we only have growth data up to 2018 to study the effect of the 2017 frost. With regard to the time period before the spring frost, we chose 6 years to buffer the effect of the other spring frosts on these indices. In this respect, the 2010 spring frost is affecting the BAI_{i-6} of the 2013 spring-frost year, which in turn affects the BAI_{i-6} of the 2017 spring-frost year. We applied the Kruskal–Wallis test by ranks for independent samples and the Conover's post-hoc test, with the Bonferroni method to adjust *P*-values, to compare the *Rt* and *Rs* means between *F. sylvatica*, *Q. petraea* and *Q. pyrenaica*.

An additional analysis of the growth reductions produced by spring frost was the superposed epoch analysis (SEA; Lough and Fritts 1987). The SEA identifies the link between discrete events and continuous time processes and tests the probability

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of such association occurring by chance. We used the double-bootstrap SEA methodology described by Rao et al. (2019) to evaluate growth departures from mean values for the years with spring frost and the 2-year pre- and post-disturbance periods. To calculate the SEA we used ring-width indices as input variable. To obtain these indices we removed biological trends of the raw tree-ring widths by calculating the detrended chronologies for each species using the ARSTAN program (Holmes 1997). We applied a double detrending procedure to remove long-term growth trends (Holmes et al. 1986). First, we fitted a negative exponential curve to raw ring-width data, to remove the effect of age and of low competition during the first years of the growth series, that is, during the establishment of the analyzed trees in the open woodland (before the cattle grazing ban). Second, we fitted a 32-year long cubic smoothing spline, to remove the low-frequency stand dynamics signals. The autocorrelation of the resulting detrended time series was removed by applying a first-order autoregressive model but reintroducing into the residual series part of the low-frequency signal in order to retain climatically- related autocorrelation in growth.

Spring frost effect on spectral phenology

The impact of frosts on spectral phenology was studied with an NDVI dataset recently developed by Vicente-Serrano et al. (2020) over the entire peninsular Spain and Balearic Islands for the period 1981–2015. This dataset is based on the National Oceanic and Atmospheric Administration–Advanced Very High Resolution Radiometer (NOAA–AVHRR) afternoon images, and has a 15-day temporal resolution, with 1.1-km^2 spatial resolution. In order to compare the annual spectral dynamics of pre-frost and post-frost periods with the frost year dynamics, we averaged the NDVI annual series of three groups of years: 1993–94, 2008–09 and 2011–12, representing the 2-year pre-frost period; 1996–97, 2011–12 and 2014–15, representing the 2-year post-frost period; and 1995, 2010 and 2013, representing the frost year. The 2017 frost period was not included in this analysis as there were not enough data available. TIMESAT software (Eklundh and Jönsson 2017) was used to determine the spectro-phenological start, length and end of the season, as well as the peak NDVI values and DOY of occurrence. The start- and end-of-season parameters were determined based on seasonal amplitude, defined as the difference between the base and maximum NDVI values for each season. The start occurs when the left part of the fitted curve reaches a percentage of that amplitude value, counted from the base level, and the end of season is defined similarly, but for the right side of the curve (see Figure 7). These values were calibrated with data from the phenological inventory. The corresponding parameters for pre-frost, frost and post-frost years were analyzed and compared. Gaussian curves were adjusted to the pre-frost, post-frost and frost series to enable comparison. Finally, we tested the differences in NDVI values for each date (averaged for each group of years) between pre-frost, frost and post-frost years with Mann–Whitney *U* tests.

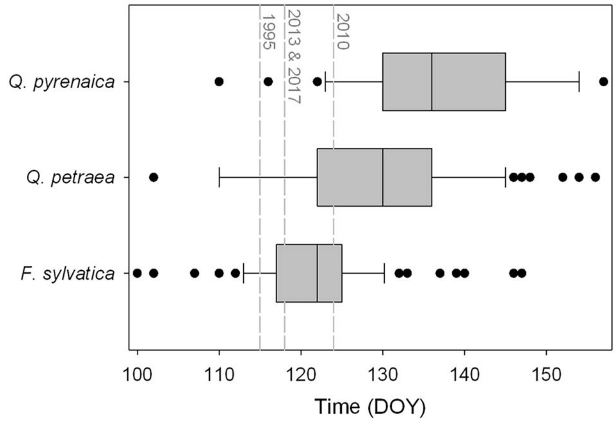

Figure 2. Box-plots for leaf emergence time (DOY) in *F. sylvatica*, *Q. petraea* and *Q. pyrenaica* monitored during the period 2006 to 2011. The spring frosts are marked with dashed vertical lines. The median, the 10th, 25th, 75th and 90th percentiles and the outliers are represented. Due to the numerous outliers the graphs are truncated on the right.

Results

Leaf emergence

Excluding the outliers (values below or above the 10th and 90th percentiles, respectively), leaf flushing started in *F. sylvatica* and *Q. petraea* almost simultaneously, on DOYs 113 and 110, respectively, whereas *Q. pyrenaica* leaf flushing started later, on DOY 123 (Figure 2). Keeping the outliers out of the analysis, the variability in leaf flushing time was higher in *Q. petraea* and to a lesser extent in *Q. pyrenaica*, than in *F. sylvatica*. That is, the leaf emergence period was longer in *Q. petraea* (35 days) and *Q. pyrenaica* (31 days) than in *F. sylvatica* (17 days). This means that the four late frosts recorded in the forest since 1994 affected a higher proportion of developing shoots in *F. sylvatica* than in *Q. petraea*, whereas *Q. pyrenaica* was hardly affected by these events. In this respect, the spring frost of 2010, which coincided with the period of leaf phenology monitoring (2006–11), occurred when ~50, 25 and 0% of the buds were opening in *F. sylvatica*, *Q. petraea* and *Q. pyrenaica*, respectively (Figure S1 available as Supplementary data at *Tree Physiology* Online).

Spring frost effects on BAI

Fagus sylvatica trees exhibited the highest variability in growth. The coefficients of variation for the growth series since 1980 were 0.280, 0.169 and 0.174 for *F. sylvatica*, *Q. petraea* and *Q pyrenaica*, respectively. *Fagus sylvatica* was also the species with the greatest growth reductions in the years with spring frost, namely 1995, 2010, 2013 and 2017 (Figure 3). These growth reductions were similar to the reduction in growth registered

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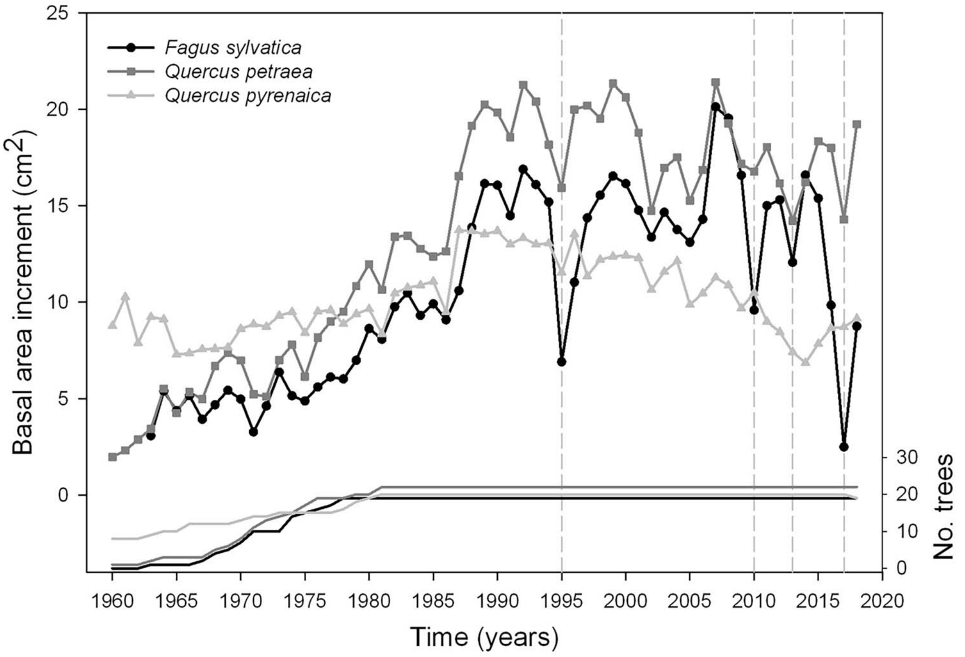

Figure 3. Basal area increment and number of cored trees with growth rings (right *y*-axis) dating back to 1960. The spring frosts are marked with dashed vertical lines. The forest is monitored since 1994, therefore possible earlier frosts are missing.

in 2016, the year with the warmest and driest summer since 1994 (see average annual climate data in Figure S2 available as Supplementary data at *Tree Physiology* Online).

Quercus petraea also exhibited a reduction in growth during the years with spring frosts, but of lower magnitude than that of *F. sylvatica*. In the spring frost year of 2010, the growth of *Q. petraea* was similar to that of the previous year (Figure 3). In addition, *Q. petraea* presented similar growth reductions during 2002, one of the coldest years of the series, and 2005, the driest year of the series (Figure S2 available as Supplementary data at *Tree Physiology* Online). In contrast, *Q. pyrenaica* showed little year-to-year growth variability during the entire series, and no remarkable growth reductions during frost years. This species showed a decreasing trend in growth since 1987, when it reached the maximum growth.

Fagus sylvatica had significantly lower frost-resistance (*Rt*) than both *Quercus* species in the years 1995, 2010 and 2017 (Figure 4). In 2013, no significant differences in resistance were found among species. Differences in resistance between *Q. petraea* and *Q. pyrenaica* were not significant in any year except for 2017. Although this result suggests that both species had a similar behavior during the frost years, the complementary SEA shows that only *F. sylvatica* and *Q. petraea* (not *Q. pyrenaica*) exhibited significant relationship between frost events and growth reductions (Figure 5).

Growth frost-resilience (*Rs*) was significantly lower in *F. sylvatica* than in both *Quercus* species after the 1995 and 2017 frosts (Figure 4), although no significant relationship was found between these values and frost events according to the SEA (Figure 5). In any case these low growth values during the years 1996 and 2018 observed in *F. sylvatica* did not seem to correspond to the climatic conditions of those years, which were cool and rainy (and potentially favorable for growth) during all or a large part of the year (see monthly temperature and precipitation of the frost and subsequent years in Figure S3 available as Supplementary data at *Tree Physiology* Online). *Quercus pyrenaica* had significantly lower *Rs* than the other two species after the 2010 and 2013 frosts (Figure 4); however, this result seemed to be more related to the decreasing growth trend of this species (Figure 3) than to the spring frosts (Figure 5).

The resistance index (*Rt*) has been calculated for the entire growth series since 1977 (Figure 6) to analyze whether there were other factors, apart from spring frosts, inducing growth drops. The most important growth drops in *F. sylvatica* occurred in years with spring frosts (2017, 1995 and 2010, ordered from the lowest to the highest resistance), while in both *Quercus*, since growth declines during these years were not so severe, the lowest resistance occurred in years other than those with spring frost (2002 for *Q. petraea* and 2014 for *Q pyrenaica*). The three tree species showed a decreasing trend in *Rt* between 1977 and 2018. The slopes of the time-resistance regressions were −0.008, −0.002 and −0.003 for *F. sylvatica*, *Q. petraea* and *Q pyrenaica*, respectively, being significant for *F. sylvatica* ($P = 0.007$) and *Q. pyrenaica* ($P = 0.027$), but not for *Q. petraea* ($P = 0.201$).

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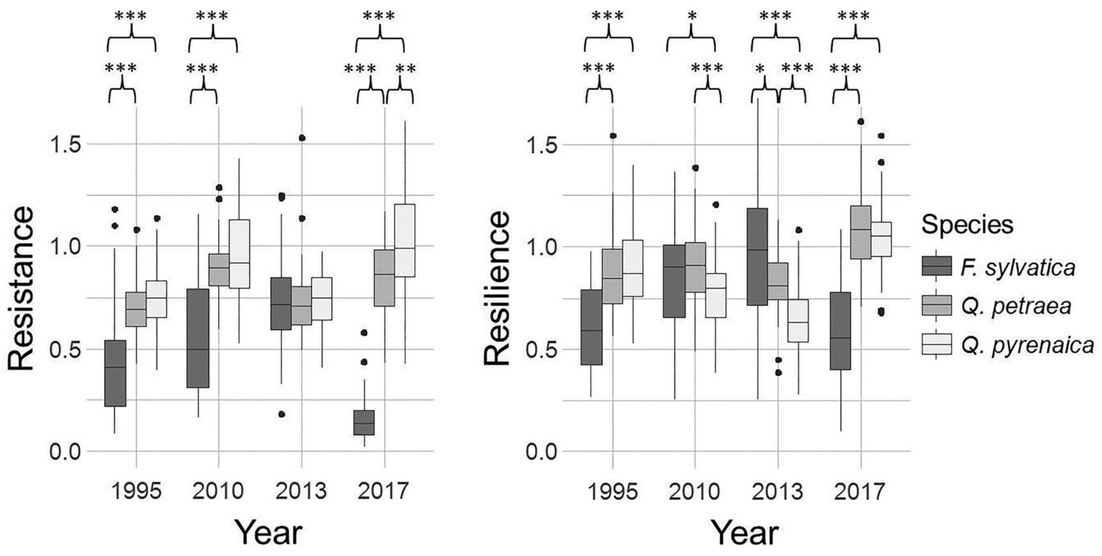

Figure 4. Resistance (*Rt*) and resilience (*Rs*) indices for *F. sylvatica* (dark-gray box-plots), *Q. petraea* (medium-gray box-plots) and *Q. pyrenaica* (light-gray box-plots) for the 4 years with spring frost recorded since 1994. Significance levels of the differences: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

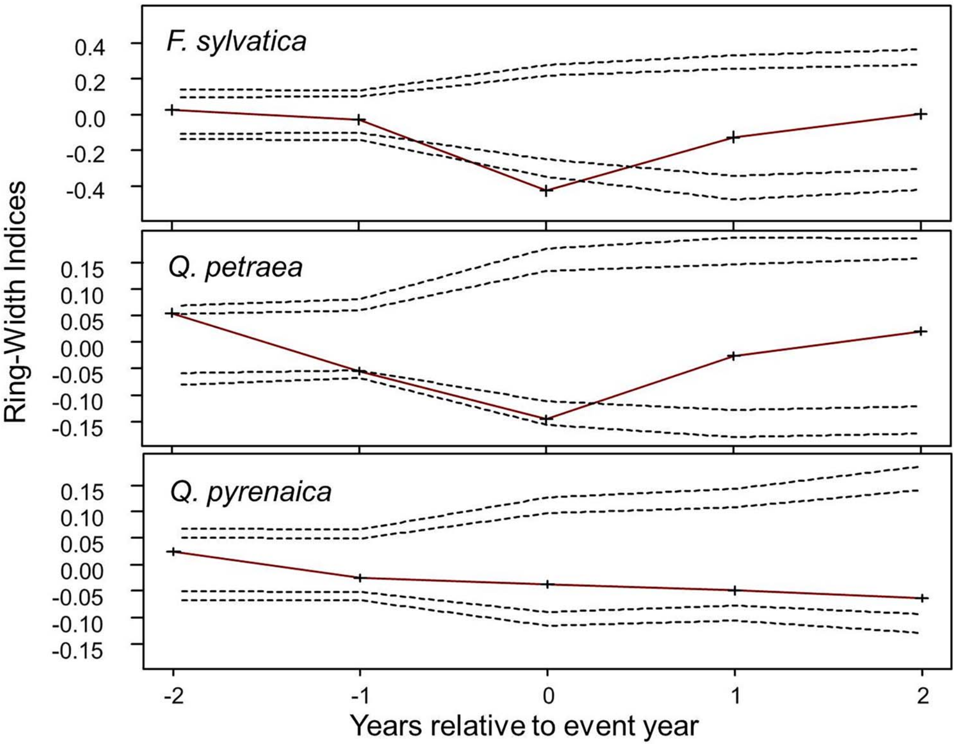

Figure 5. Superposed epoch analyses showing positive and negative growth departures from mean growth values for the year with frost damage (year 0) and 2 years before (years −1 and −2) and after this event (years +1 and +2). Values were calculated considering the 4 years with frost damage (1995, 2010, 2013 and 2017). The dashed lines denote the thresholds of significance ($P < 0.05$ and $P < 0.01$).

Spring frost effect on spectral phenology

There were no significant differences in the maximum NDVI values between the years in which the spring frost occurred and the pre- and post-frost years. The length of the spectro-phenological season (time period between sprouting and senescence of leaves as determined from NDVI values) was 168 days for pre- and post-frost years and 196 days for frost years (Figure 7). In pre-frost years, the start and end of the spectro-phenological

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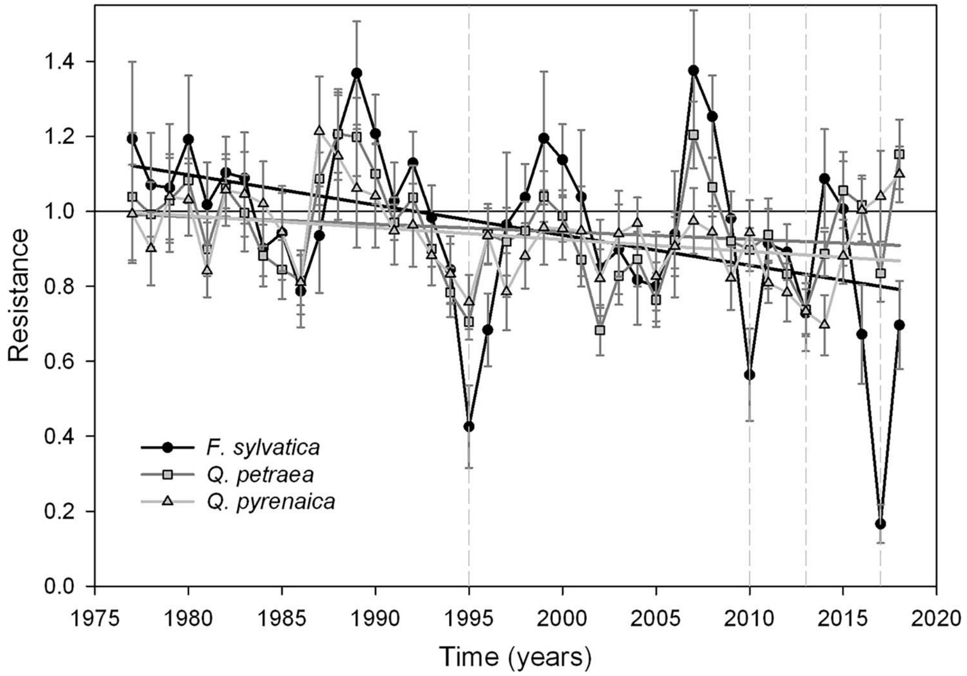

Figure 6. Trends in resistance index (*Rt*; i.e., the BAI in a given year divided by the mean BAI over the 6 previous years) since 1977. Data are means and 95% confidence intervals per species. Linear regressions were fitted to each species. The spring frosts are marked with dashed vertical lines.

season occurred on DOY 113 and 281, respectively; in frost years it occurred on DOY 127 and 323, respectively; and in post-frost years it occurred on DOY 127 and 295, respectively. Thus, both the beginning and end of the spectro-phenological season occurred earlier in pre-frost years than in frost years, with intermediate behavior in the post-frost years. Similar performance occurred with the timing of NDVI peak which occurred on DOY 169, 190 and 183, for the pre-frost, frost and post-frost years, respectively. In any case, none of these differences were statistically significant.

Discussion

Large stem growth reductions caused by spring frosts in F. sylvatica, not in Q. pyrenaica

Spring frosts caused high growth reductions in *F. sylvatica*, moderate growth reductions in *Q. petraea* and no significant growth reductions in *Q. pyrenaica* (Figures 3 and 5). Based on visual observations of leaf malformation and scorching after spring frosts, and the negative relationship between frost-induced growth reduction and leaf flushing time across species, we suggest that leaf phenological stage (and associated probability of frost leaf damage) is a major determinant of reduced growth during frost years, and sometimes the year afterwards. This hypothesis is reinforced by the results obtained in Allevato et al. (2019), where damage severity in beech trees was related with the phenological stage.

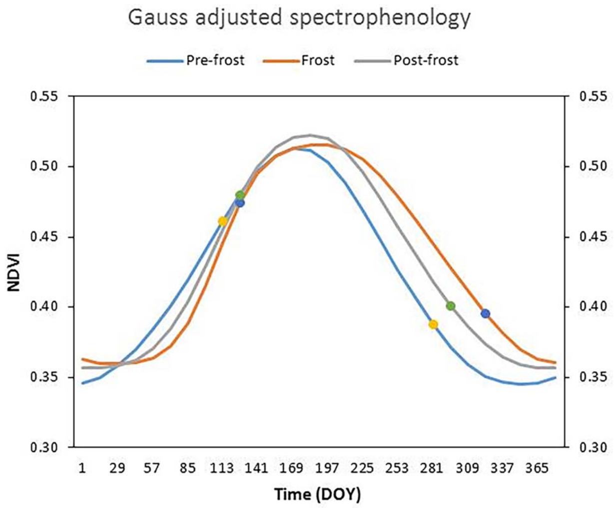

Figure 7. Gaussian model of the intra-annual NDVI time series in the years with frost damage (1995, 2010 and 2013; 2017 was not included due to lack of data), in the 2 years prior to frost years and in the 2 years after frost years. The start and end of the spectro-phenological season are marked with dots.

According to previous studies, the freezing temperature of the leaves of *F. sylvatica* and *Q. petraea* is around −5 °C (Lenz et al. 2013); to our knowledge no work has studied the freezing temperature of *Q. pyrenaica* leaves. However, there are other factors influencing leaf damage by frost, such as air relative humidity, wind, prior temperature conditions, rate of temperature drop and, especially, the duration of the frost (Min

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et al. 2020). Indeed, the four spring frosts recorded in the study forest reached temperatures between −2.0 and −4.6 °C, and were preceded by weekly warm periods (Figure 1).

Meteorological conditions preceding spring frosts may condition frost impact on the leaves and on stem growth. In *F. sylvatica*, the largest growth declines occurred in 1995 and 2017 (Figure 4), which had warmer winters and springs than normal (Figure S3a available as Supplementary data at *Tree Physiology* Online), 2017 being the warmest year in the series (Figure S2 available as Supplementary data at *Tree Physiology* Online). Warmer late-winter temperatures would induce an earlier leaf emergence (Vitasse et al. 2009) and, conversely, in cold years such as 2010 (Figure S3a available as Supplementary data at *Tree Physiology* Online), leaf emergence would be delayed (compare Figure 2 with Figure S1 available as Supplementary data at *Tree Physiology* Online). Therefore, for the same frost date (all spring frosts studied in this work occurred on a very similar date, April 28 ± 5 days), the impact is greater in warm years with advanced phenology, that is, with a higher proportion of buds sprouted and therefore susceptible to being affected by frost. The relationship between high temperatures leading to advanced phenology and frost damage, which has also been shown by Sangüesa-Barreda et al. (2021), can lead to confounding the effects of spring frosts with those of droughts, and to erroneous conclusions on the factors that control growth when the spring frost events are unknown and not analyzed. This may be the case for the growth drops of 1995 and 2017, when drought effect would be secondary, since drier years with similarly high temperatures such as 2015 (Figure S2 available as Supplementary data at *Tree Physiology* Online) did not produce a growth decrease in *F. sylvatica*. Despite leaf phenology of *Q. petraea* being more temperature-dependent than that of *F. sylvatica* (Vitasse et al. 2009), the impact of spring frosts on growth was greater in *F. sylvatica* (Figure 4), making this species more vulnerable to these extreme climate events. The lower impact of spring frosts on *Q. petraea* growth is partly explained by the lower damage suffered by the leaves, which would be in turn related to its later leaf phenology (Figure 2), but it is also explained by the differences between tree species when faced with the same damage. In this sense, the loss of a cohort of leaves caused by a spring frost seems to produce a decrease in growth in *F. sylvatica* but not in *Q. petraea* (Rubio-Cuadrado et al. 2021). The lower *Q. petraea* growth produced in frost years (Figure 5) seems to be due mostly to a delay in phenology, possibly caused by the freeze–thaw events (Tedla et al. 2020), rather than to a direct effect of the damage suffered by the frozen leaves. Effectively, according to a previous study conducted in the same area (Rubio-Cuadrado et al. 2021), the *F. sylvatica* trees damaged by the 2017 frost due to their earlier leaf flushing lost the first cohort of leaves, suffered a great delay in the emergence of the second leaf cohort and, consequently, reduced their growth drastically compared with those undamaged, with later leaf flushing. However, undamaged and damaged *Q. petraea* trees showed similar radial growth increments. Although the damaged *Q. petraea* trees also lost the first cohort of leaves, both undamaged and damaged trees suffered a similar delay in the emergence of the first and second leaf cohorts, respectively. The differences between tree species in the response to the same damage may be related to the fact that wood formation in *F. sylvatica* generally begins after budburst and use mostly carbon assimilated by the leaves of the current year, with a maximal growth rate when the leaves are mature (Čufar et al. 2008, Michelot et al. 2012), whereas in ring-porous species such as *Q. petraea*, wood formation begins before budburst using carbon reserves (Michelot et al. 2012, Gričar et al. 2017, Puchałka et al. 2017). Regarding *Q. pyrenaica*, it did not suffer any significant drop in growth compared to the years without spring frost (Figure 5) due to its much more delayed phenology (Figure 2 and Figure S1 available as Supplementary data at *Tree Physiology* Online).

Ice formation in the xylem can occur at the relatively high subfreezing temperatures registered in the four spring-frost years (−2 to −4.6 °C), particularly in young and large xylem conduits (Davis et al. 1999, Neuner et al. 2010). Hence, a priori, the larger xylem vessels of *Q. petraea* and *Q. pyrenaica* would make the xylem of these species more sensitive to freezing than that of *F. sylvatica* (Davis et al. 1999, Cavender-Bares 2005). However, our results suggest that differences in leaf phenology between species (earlier in *F. sylvatica*) are more important drivers of the extent of growth reductions caused by spring frosts than differences in xylem vessel diameter and theoretical xylem vulnerability to freeze–thaw embolism (lower in *F. sylvatica*).

Drought effects on growth were less noticeable than those of spring frosts, especially in *F. sylvatica*. Given that 'El Hayedo de Montejo' is one of the southernmost European forests where *F. sylvatica* and *Q. petraea* coexist, and knowing that these species, especially *F. sylvatica*, are drought sensitive (Scharnweber et al. 2011), we expected to find important growth reductions due to droughts (Hampe and Petit 2005, Hanewinkel et al. 2013), as has been found in other nearby similar forests (Rubio-Cuadrado et al. 2018*a*) and also in northern populations subjected to a more humid climate (Scharnweber et al. 2011, Vannoppen et al. 2020). However, the main growth reductions in *F. sylvatica* were due to spring frosts (Figures 3 and 6), 2016 being the only year in which the growth decline in *F. sylvatica* seemed to be related to drought (Figure S2 available as Supplementary data at *Tree Physiology* Online). Although rainfall was very low during summer months for the 1994–2018 period (31 mm on average), leaf predawn water potential, a surrogate of soil water availability to plants, was rarely below −0.5 MPa in *F. sylvatica* and −1 MPa in *Q. petraea* and *Q. pyrenaica* adult trees during summer in the study area (Cano et al. 2013,

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Rodríguez-Calcerrada et al. 2019). This is possibly due to high soil water retention and tree rooting depth in the study site. In any case, previous studies have shown a reduced impact of summer drought on the growth of southern beech forests, probably resulting from a shortening of the radial growth period, so that most of the growth is concentrated in the wettest period (spring and early summer), thus reducing the possible impact of summer droughts (D'Andrea et al. 2020, 2021). These results would confirm that trees in rear-edge populations can display a high resistance to drought (Cavin and Jump 2017, but see Camarero et al. 2021), although they could be particularly vulnerable to other climate extremes, such as spring frosts, particularly under continental conditions. In *Q. petraea*, only in 2005 did the reduction in growth coincide with a severe summer drought; the rest of growth reductions coincided with spring frosts or, in 2002, with a possible reduction in the vegetative period related to low temperatures (Figure S2 available as Supplementary data at *Tree Physiology* Online). *Quercus pyrenaica* showed very low sensitivity to both dry years and spring frosts years, which suggests a better adaption to the Mediterranean mountain climate. The negative growth trend since 1987 observed in this species is due to the increase in competition that has occurred in the study forest in recent decades, which would favor the more competitive *F. sylvatica* (Rubio-Cuadrado et al. 2020*a*).

Frost legacies: decreasing resistance index and delayed senescence

The growth resilience after the spring frosts of 1995 and 2017 was significantly lower in *F. sylvatica* than in both *Quercus* species (Figure 4). Although there was no statistically significant relationship between the spring frosts and the *F. sylvatica* growth declines of 1996 and 2018 based on the SEA (Figure 5), these declines are difficult to explain only by the climate of these years, with cool temperatures and higher winter and spring rainfall than average (see resistance index in Figure 6, and climate in Figure S3c and d available as Supplementary data at *Tree Physiology* Online). A legacy of spring frosts on growth was observed in a previous work performed in the same forest (Dorado-Liñán et al. 2017). In this work, centennial *F. sylvatica* trees exhibited a growth reduction after the 1995 frost that had not yet reversed in 2013. Compared with our results, obtained in 50-year-old *F. sylvatica* trees (Table S1 available as Supplementary data at *Tree Physiology* Online), the reduction in growth for almost a decade in larger, centennial *F. sylvatica* trees suggests a different frost legacy on growth depending on tree size or age, which should be further investigated. In any case, this age effect does not seem to be generalizable (Jiao et al. 2020), and there may be other factors that condition it, such as the effect of past land use or the vigor decline after a certain age.

Since the resistance index (*Rt*) is a relative and dimensionless index, it is not expected to find any trend in its dynamics. This index has not changed over the last decades in *Q. petraea*, but it has decreased in *Q. pyrenaica* and *F. sylvatica* (Figure 6). For *Q. pyrenaica*, this result is probably due to its negative growth trend (Figures 3 and 6) associated with increasing competition (Rubio-Cuadrado et al. 2020*a*). However, for *F. sylvatica*, there was no significant effect of competition on growth (Rubio-Cuadrado et al. 2020*a*). Therefore, the reduced *Rt* in recent decades is likely a legacy of spring frosts. The legacies of extreme climate events on growth have been attributed mainly to physiological factors, especially to damages in the hydraulic system and limitations in the availability of non-structural carbohydrate reserves (Hacke et al. 2001, Adams et al. 2017, Timofeeva et al. 2017, Schönbeck et al. 2018, Peltier and Ogle 2019). In line with this, the availability of non-structural carbohydrates is probably not a reason for lower resilience (*Rs*) of *F. sylvatica*. Previous studies showed a reduced impact of late frosts on non-structural carbohydrate concentrations of *F. sylvatica* (Rubio-Cuadrado et al. 2021); when a significant impact occurs this is rapidly reversed (D'Andrea et al. 2019, 2021). In addition, the lower proportion of parenchyma and non-structural carbohydrate concentrations in the sapwood of *F. sylvatica* as compared with that of the two *Quercus* species may be compensated by a higher sapwood depth (Rodríguez–Calcerrada et al. 2015). In any case, the decreasing trend of *F. sylvatica* growth may be explained by several causes. First, the increase in spring frost frequency itself and its cumulative effects on *Rt*; that is, the accumulation of late frosts at the end of the period considered (1994–2018), which influences the slope of the regression line between time and *Rt*. Second, the greater impact of the 2017 spring frost resulted from both the lower temperatures reached during the frost days (Figure 1) and the fact that it was the warmest year in the series (Figures S2 and S3 available as Supplementary data at *Tree Physiology* Online), which would increase the number of sprouted buds when the frost occurred. Third, the occurrence of spring frosts on overlapping recovery times, which can potentially affect ecophysiological memory, amplifying a negative carbon balance or damaging the xylem and reducing hydraulic conductivity (Szejner et al. 2020). Lastly, fourth, the increase in temperatures and the decrease in precipitation that have occurred in the last decade (Figure S2 available as Supplementary data at *Tree Physiology* Online) may have magnified the negative effect of spring frosts due to increasing drought stress.

The spatial resolution of the NDVI dataset employed (1.1 km^2) prevents obtaining detailed information to distinguish the frost effects on different species, but other factors affecting species phenology and frost impact, such as the aspect or altitude (Allevato et al. 2019) are fairly homogeneous in the study area (see section Study area). The information obtained should be understood as representative of the overall response

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of the forest, where the NDVI increases gradually as the different species sprout: early sprouting species should determine the start of the steep rise of NDVI whereas the late sprouting determines the end. The NDVI dataset shows another legacy of the spring frost, a delay in autumn leaf senescence in the frost year and also but less markedly in the following year (Figure 7). This trend is consistent with a previous study conducted in the same forest about the consequences of the 2017 frost on stem, shoot and leaf functional traits, including leaf phenology using shorter NDVI time series, but with higher temporal and spatial resolution (Rubio-Cuadrado et al. 2021). Here, we found no significant differences in NDVI between years, but this is partly because of the low number of spring-frost years with NDVI data (1995, 2010 and 2013) and the low spatial resolution of the dataset analyzed. Large-scale studies performed in the Northern Hemisphere have shown a significant positive relationship between the timing of leaf flushing and leaf senescence in ~20% of the studied areas (Liu et al. 2016). Temperate, mixed beech-oak forests would be one of the forest types in which this relationship would be particularly strong (Fu et al. 2014). To our knowledge, there are no studies reporting frost legacy effects on stem growth or leaf phenology. However, such effects are possible, given the relationship between leaf life span, growth and carbon gain and frost effects on all three variables during the frost year (Rubio-Cuadrado et al. 2021). More detailed research is needed on this subject.

Management implications

In forests where *F. sylvatica* coexists with oaks, beech trees tends to outcompete the other species (Rohner et al. 2012, Petritan et al. 2017, Rubio-Cuadrado et al. 2018*b*) due to its greater competitiveness and shade tolerance (Hein and Dhôte 2006, Manso et al. 2015). Likewise, in the studied forest, *Q. pyrenaica* is the most abundant species of the upper stratum (measured in basal area), but *F. sylvatica* dominates in the proportion of seedlings and saplings (Vélez Olalde 2016). However, *F. sylvatica* was more sensitive to spring frosts and, according to Sangüesa-Barreda et al. (2021), the frequency and geographic extent of spring frost events affecting this species has increased rapidly in the last two decades in southern Europe. If this trend continues, *F. sylvatica* may see their development compromised by more recurrent climatic extreme events. Therefore, the foreseeable retraction of oaks in benefit of *F. sylvatica* may bring about a loss of ecosystem resilience. Management strategies aimed at maintaining a mixed stand with diverse light-regeneration niches to favor the presence of *F. sylvatica* and both oaks species in the understory should be considered as a means to promote forest resilience. In addition, promoting the regeneration of *F. sylvatica* phenotypes showing late leaf emergence could improve the resilience of this species to spring frosts.

Conclusions

In a mixed beech-oak rear-edge population, *F. sylvatica* showed a decades-long trend of decreasing resistance of growth and higher growth sensitivity to spring frosts than two co-occurring oak species (*Q. pyrenaica* and *Q. petraea*), suffering severe radial growth reductions, often stretched over 2 years, after the occurrence of the spring frosts. Leaf flushing time (earlier in *F. sylvatica*) and leaf damage (more frequent and severe in *F. sylvatica*) appeared to be more determinant factors of stem growth reduction during the spring-frost year than stem xylem anatomy, based on the consideration that narrower vessels of *F. sylvatica* are, a priori, less vulnerable to freeze–thaw embolism. Spring frosts caused significant growth reductions in *Q. petraea* trees too, although of lower magnitude and only during the year of the spring frost. By contrast, the spring frosts did not affect *Q. pyrenaica* growth. Although the studied forest is located at the southernmost distribution limit of *F. sylvatica* and *Q. petraea*, the impact of spring frosts on their growth has been considerably greater, especially in *F. sylvatica*, than the drought impacts. Taking into account the expected increase in the frequency of these events in Europe, spring frosts may become one of the greatest hazards for these rear-edge *F. sylvatica* populations.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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