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Long- and short-term impacts of a defoliating moth plus mistletoe on tree growth, wood anatomy and water-use efficiency

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ABSTRACT

Climate warming and biotic stressors are expected to reduce tree radial growth and performance at short and long time scales. However, the impacts of different biotic stressors on performance throughout a tree's life are largely understudied. Here we assessed the effects of a past nun moth (*Lymantria dispar*) outbreak and related defoliation on Scots pine (*Pinus sylvestris*) trees, which were later severely infested by the hemiparasite mistletoe (*Viscum album* subsp. *austriacum*). We compared the responses of trees severely infested or not infested by mistletoe in a wet vs. a dry site to quantify the relative importance of biotic stressors under different climate conditions. We used dendrochronology to quantify: long- and short-term changes in radial growth (resilience), differences in wood anatomy during the outbreak, and recent changes in intrinsic water-use efficiency (*WUEi*). The outbreak caused a sharp growth reduction in 1953 (50% decrease in basal area increment –BAI) and the formation of tracheids of small transversal lumen diameter (33% decrease in diameter). Recent mistletoe infestation caused a difference in growth between infested and non-infested trees lasting 34 and 21 years in the wet and dry sites, respectively. Growth (BAI) decreased more steeply in severely infested than in non-infested trees, the post-drought resilience decreased in severely infested trees, and the *WUEi* increased, particularly in the dry site. The BAI of severely infested trees was more negatively impacted by warm and dry conditions during the growing season than in non-infested trees, particularly in the dry site. Tree rings recorded historical effects of biotic stressors (*L. monacha* outbreak), which may constrain responses to recent stressors (mistletoe).

1. Introduction

Some biotic stressors (e.g., hemiparasitic plants, herbivorous insects, or pathogens) may benefit from climate warming, which enhances their reproduction, survival and growth rates (Ayres and Lombardero, 2000). Climate variability can alter host-stressor relationships by expanding the distribution of biotic stressors and increasing their infestation rates (Sangüesa-Barreda et al., 2018), or making them more harmful to trees (Sturrock et al., 2011). However, few studies have considered the successive effects of biotic stressors on tree performance and reconstructed their impacts through the tree lifespan.

The mistletoe (*Viscum album* subsp. *austriacum* (Wiesb.) Vollmann, hereafter mistletoe) is a major stressor of forests since this hemiparasitic plant obtains water and nutrients from their tree hosts (conifers), but it also photosynthesizes (Zuber, 2004; Glatzel and Geils, 2009). In this species, a recent upward shift in altitude has been reported in response to successive warmer winters (López-Sáez, 1993; Dobbertin et al., 2005), and its altitudinal distribution could further change if warming continues (Zamora and Mellado, 2019). Since mistletoe is a xylem-tapping species it continues transpiring under dry conditions and this increases the host drought stress further (Fischer, 1983; Sangüesa-Barreda et al., 2012; Zweifel et al., 2012). In seasonally dry sites, severely infested host trees show crown defoliation (Rigling et al., 2010), leading to a reduction in radial-growth rates, and thus causing a loss in forest productivity (Hawksworth, 1983; Noetzli et al., 2004; Mutlu et al., 2016).

In drought-prone Mediterranean Scots pine (*Pinus sylvestris* L.) forests, located near the species southernmost distribution limit, the combined negative effects of mistletoe and severe droughts reduced

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growth, and raised mortality rates (Sangüesa-Barreda et al., 2012, 2013). These studies indicated that the host-mistletoe interaction responded to climate variability, and contributed to explain forest dieback in combination with drought stress. In fact, a worldwide study demonstrated that mistletoes and hosts have similar responses to precipitation gradients regarding water and nitrogen use (Scalon and Wright, 2015). Therefore, a lower reduction in growth rates of infested trees could be expected in wet sites or periods, whereas growth loss could be amplified by dry conditions due to a higher mistletoe demand for water.

Trees currently infested by mistletoe may have been affected by biotic stressors in the past including insect defoliators as *Lymantria monacha* ('nun moth'), whose outbreaks cause leaf loss, growth reduction, and death in several tree species including pines (Bejer, 1988). Here, we address the study of the effects on tree growth and performance of two biotic stressors, the mistletoe, which is causing decline of some drought-prone Scots pine forests in Europe (Rigling et al., 2010), and *L. monacha*, a major insect defoliator affecting European conifers. We capitalize on a severe outbreak of *L. monacha*, which affected Scots pine forests from central and eastern Spain during the 1920s and 1950s causing widespread defoliation in some stands (Rupérez, 1958). Nowadays, some of these forests also show high levels of mistletoe infestation (Sangüesa-Barreda et al., 2012). Using dendrochronology, we aimed to: (i) characterize and reconstruct the radial-growth patterns (trends, post-drought resilience), and responses to climate of Scots pine trees showing different levels of recent mistletoe infestation; and (ii) to relate it with the potential growth loss caused by the past *L. monacha* outbreaks observed in the 1920s and 1950s. We compared growth responses in a wet vs. a dry site to discern the relative importance of climate stress. We also quantified changes in wood anatomy (transversal lumen diameter, cell-wall thickness) after the 1950s outbreak, and recent changes in intrinsic water-use efficiency (*WUEi*) to understand how the two biotic stressors (mistletoe, defoliator insect) have affected tree growth and water use. The *WUEi* is the ratio between the C assimilation rate (A) and the stomatal conductance rate (g_s) and allows estimating the trade-off between CO_2 uptake and water loss through stomata (Farquhar et al., 1982). For instance, an increase in *WUEi* may reflect reduced water loss due to stomata closure for the same C uptake. We hypothesize a long-term growth reduction, and a recent increase in *WUEi* of severely infested trees as compared to non-infested trees, particularly in the driest site, and a short-term growth reduction and changes in anatomy (decrease in number and transversal lumen diameter of tracheids) due to the defoliation and related to the 1950s insect outbreak.

2. Materials and methods

2.1. Study sites

The two study sites (Noguera de Albarracín –hereafter abbreviated as Noguera, 40° 28′ N, 1° 38′ W, 1520 m a.s.l.; Manzanera, 40° 01′ N, 0° 38′ W, 1562 m a.s.l.) are located in Teruel, Aragón (eastern Spain) (Fig. 1). The study sites experience Mediterranean, continental climate conditions with cold winters and dry summers (see Camarero et al., 2016). Mean annual temperature varies between 8.9 °C (Noguera) to 11.5 °C (Manzanera), and annual precipitation ranges between 620 mm (Noguera) and 475 mm (Manzanera). Therefore, Manzanera can be considered a warmer and drier site than Noguera. Soils are loamy, usually acid and of the cambisol type (see Gazol et al., 2018a). The Scots pine basal area is 33.8 and 25.7 $m^2 ha^{-1}$ in Noguera and Manzanera, respectively. In this area, Scots pine (*Pinus sylvestris* L.) forms pure forests with the understorey dominated by *Juniperus communis* L., and *Berberis vulgaris* L. Both stands show an important presence of mistletoe since at least the last 30 years (MMA, 2006).

2.2. Insect outbreak

A severe outbreak of *Lymantria monacha* L. (Lepidoptera) was reported in the study area in the 1920s (peaking in 1926), and in the late 1940s, and 1950s (peaking in 1948–1953) (Rupérez, 1958). This insect defoliator is widely distributed across Eurasia and its moths feed on leaves and buds during spring affecting conifers (e.g. Scots pine) but also hardwood species (e.g., oaks, European beech). Outbreaks of this insect may last three to five years in pine forests according to Humphreys and Allen (2002). The *L. monacha* larvae feed on leaves of host trees and can severely defoliate them, which results in significant growth reduction, and increased tree mortality (Kimoto and Duthie-Holt, 2006).

2.3. Field sampling

At each site, we delimited a square plot (40 m x 40 m) covering a homogeneous area where the Scots pine forest showed coexisting mature trees with diverse levels of mistletoe infestation. In the most affected patches, ca. 30–35% of the basal area corresponded to severely infested trees (J.J. Camarero, *personal observation*). All mature trees located within each plot were mapped, and their size (Dbh, diameter at breast height measured at 1.3 m; total height) was measured.

To assess the degree of mistletoe infestation, we used the classification proposed in Sangüesa-Barreda et al. (2012) and based on Hawksworth (1977), who established a semi-quantitative and visual assessment of mistletoe abundance by dividing the tree crown in three parts, i.e. considering the lower, middle and upper crown. Each crown part was rated as a function of the mistletoe presence (1) or absence (0). Finally, the degree of mistletoe infestation (ID) was obtained summing the values of the three segments and rating: ID1 trees without mistletoe (sum of the segments equal to 0), ID2 moderately infested (1–2), and ID3 severely infested trees (3). Since the first two classes showed similar growth rates (see Figure S1 in Supporting information) we based our comparisons in two classes: not infested (ID1 and ID2) vs. severely infested trees (ID3). Severely infested trees were alive when sampling was performed, and often presented a dead stem apex.

2.4. Climate data

To model radial growth as a function of monthly climate data (mean maximum and minimum temperatures and total precipitation) for trees with different degree of mistletoe infestation we used the CRU TS3.10 dataset, which is gridded at 0.5° resolution (Harris et al., 2014). Climate data were extracted from the grids delimited by coordinates 40.0–40.5 °N, 1.5–2.0 °W, and 40.0–40.5 °N, 0.0-0.5 °W in Noguera and Manzanera, respectively. Since most climate records from the local meteorological stations are short and contain gaps we only considered CRU climate data from 1950 onwards.

2.5. Dendrochronology

Wood cores were mounted, air dried and carefully sanded with progressively finer sandpapers until tree rings were identifiable (Fritts, 2001). Ring widths were measured at 0.01 mm resolution using a LINTAB measuring device (Rinntech, Heidelberg, Germany). Visual cross-dating was performed following Yamaguchi (1991), and then it was checked using the program COFECHA (Holmes, 1983). Tree ring widths were transformed to basal area increment (BAI_t) assuming a circular shape of the stems, and using this formula:

$$BAI_t = \pi \left(r_t^2 - r_{t-1}^2\right) \quad (1)$$

where r_t is the cumulative radius of the growth year and r_{t-1} is the radius of the previous year. BAI values were calculated for each core and then averaged for each tree and for each mistletoe infestation class within

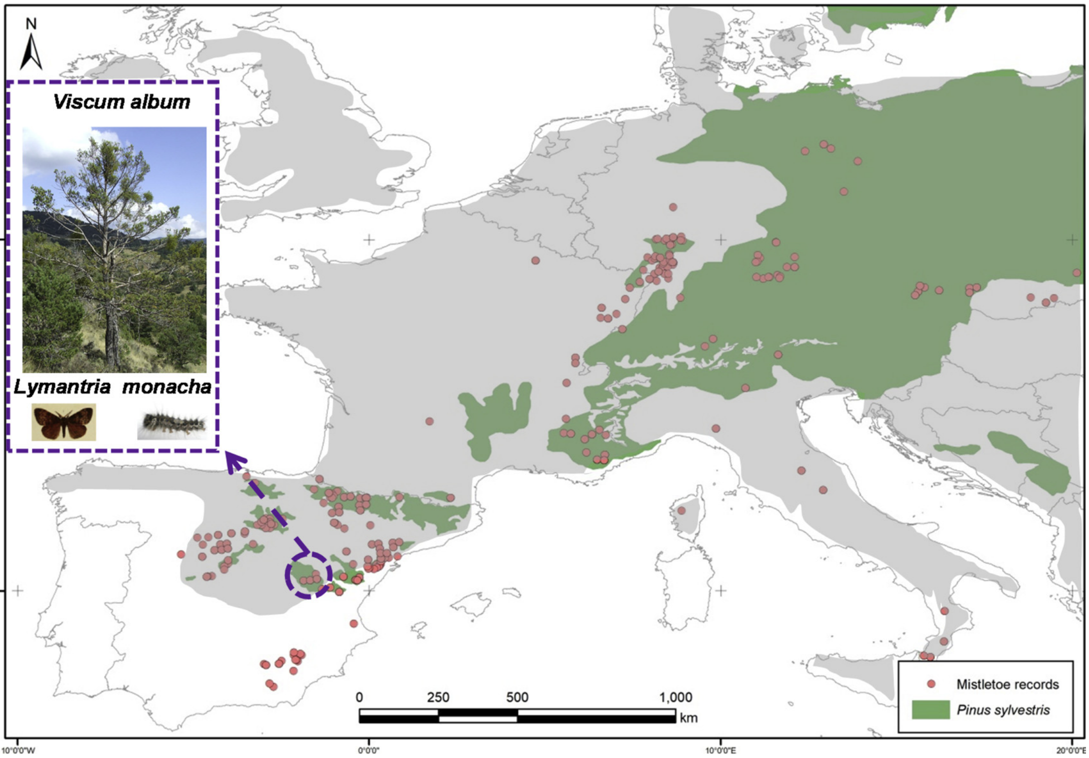

Fig. 1. Location of the study area (dashed circle) in Teruel (eastern Spain). Distribution of Scots pine (*Pinus sylvestris*, green patches) and *Lymantria monacha* (grey patches) in Europe, and mistletoe (*Viscum album*) records (obtained from Sangüesa-Barreda et al., 2018). The inset shows images of a Scots pine infested by mistletoe and *Lymantria monacha* larvae and moth.

each site.

Following Gazol et al. (2018b), we calculated short- and long-term growth responses to the *L. monacha* outbreak in the 1950s and to mistletoe infestation. To quantify long-term responses, we calculated the slopes of individual BAI values considering two 21-year periods, 1953–1973 and 1991–2011, corresponding respectively to the *L. monacha* outbreak and to recent mistletoe infestation, respectively. High and low slopes would indicate high and low long-term recovery rates in growth, respectively.

To obtain comparable metrics of resilience both the disturbance impact and the recovery rate should be considered, and referenced to a measure characterizing an undisturbed state of the analyzed system (Ingrisch and Bahn, 2018). To quantify short-term responses, we calculated a relative resilience index by quantifying the relative growth deviation (RGD) following Pimm (1984):

$$RGD = (BAI_{post} - BAI_{3\text{-yr}})/(BAI_{event} - BAI_{3\text{-yr}}) \quad (2)$$

where BAI_{post} is the BAI value in a post-event year (after the outbreak or after a drought during the infestation period), $BAI_{3\text{-yr}}$ is the BAI average for three years previous to the considered event or disturbance, and BAI_{event} is the BAI value in the event year. High and low RGD values indicate low (post-event BAI has not surpassed the BAI 3-year average) and high (post-event BAI has surpassed the BAI 3-year average) resilience, respectively. In the case of the *L. monacha* outbreak, 1953 was considered the event year, i.e. when radial growth was most impacted. In the case of mistletoe infestation, 2005 was considered the year of maximum growth loss because it was the driest year after 2000 (see Fig. 2), and mistletoe infestation was assumed to be high; i.e. the 2005 ring was a combined response to drought and mistletoe effects. Selecting the year 2005 and the 3-year interval is justified for three reasons: (i) most mistletoe individuals (> 75%) are younger than 10 years in the study Scots pine forests (Sangüesa-Barreda et al., 2012), (ii) mistletoe infestation amplifies the short-term, negative impacts of drought on growth (Sangüesa-Barreda et al., 2012), and (iii) a 3-year period has been shown to adequately assess growth responses to drought in similar forests (Gazol et al., 2018b).

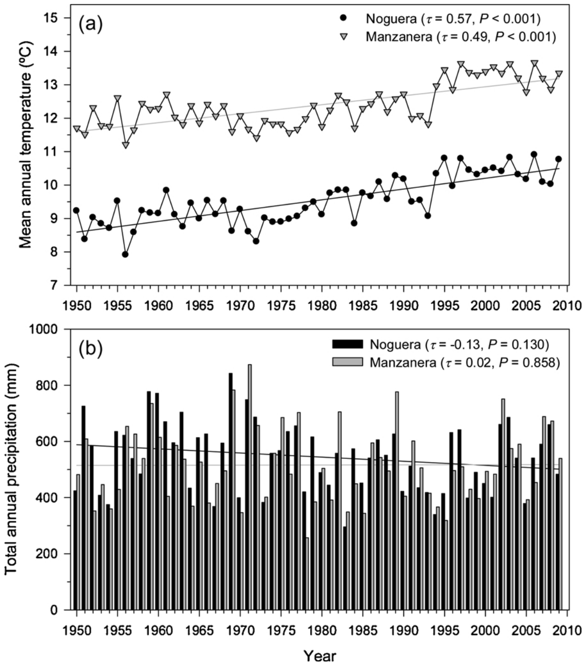

Fig. 2. Annual mean temperature (a), and total precipitation (b) estimated for the two study sites. Trends for each variable were calculated using the tau statistic (τ) its corresponding probability values (*P*).

2.6. Wood anatomy

On five cores from five trees sampled in the Noguera site and

Table 1
Characteristics of the sampled Scots pine trees as a function of mistletoe infestation. Values are means ± standard errors. Different letters indicate significant ($p < 0.05$) differences according to Mann-Whitney tests.

Study site	Mistletoe infestation class	Diameter at breast height (cm)	Height (m)	Age at 1.3 m (years)	Basal area increment of the ten most recent rings (cm^2)
Noguera (wet site)	Non-infested trees	29.3 ± 1.4	13.2 ± 0.2a	134 ± 6	8.72 ± 1.70b
	Severely infested trees	31.0 ± 1.1	14.6 ± 0.3b	141 ± 7	2.49 ± 0.67a
Manzanera (dry site)	Non-infested trees	31.9 ± 1.4	12.8 ± 0.2a	123 ± 5	6.31 ± 0.97b
	Severely infested trees	29.8 ± 1.5	14.0 ± 0.3b	116 ± 4	4.04 ± 1.03a

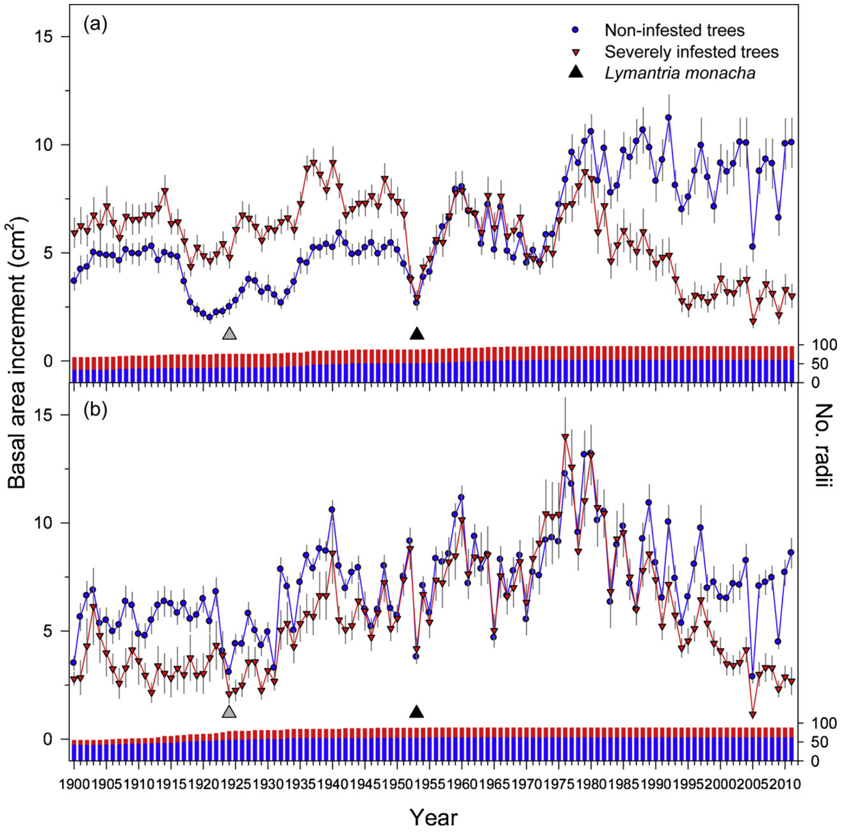

Fig. 3. Basal area increment data obtained from Scots pines in the Noguera (a, wet site) and Manzanera (b, dry site) study sites from 1900 to 2011, and considering trees without (blue symbols) or with (red symbols) severe mistletoe infestation. The upwards triangles indicate potential (1921–1924, grey upward triangles) and observed (1953, black upward triangles) *Lymantria monacha* outbreaks. Growth values are mean annual basal area increment ± SE. The right y axes show the sample size (number of measured radii, bars) with the same fill colors as the tree infestation classes.

Table 2
Measures of long- (slope) and short-term (RGD –relative growth deviation) growth resilience in Scots pine. They were calculated after a L. *monacha* outbreak (with a growth reduction occurring in 1953), and considering two levels of mistletoe infestation for the 1991–2011 period. High and low RGD values indicate low and high resilience, respectively. Values were calculated on tree basal area increment, and they are presented as means ± standard errors. Different letters indicate significant ($p < 0.05$) differences between the two infestation classes according to Mann-Whitney tests.

Study site	Mistletoe infestation class	Slope 1953–1973 ($cm^2\ yr^{-1}$)	Slope 1991–2011 ($cm^2\ yr^{-1}$)	RGD 1953 (outbreak)	RGD 2005 (mistletoe-drought)
Noguera (wet site)	Non-infested trees	0.02 ± 0.02a	0.01 ± 0.01b	0.43 ± 0.14a	0.27 ± 0.07a
	Severely infested trees	0.02 ± 0.02a	−0.05 ± 0.04a	0.52 ± 0.11a	0.55 ± 0.12b
Manzanera (dry site)	Non-infested trees	0.03 ± 0.02a	−0.03 ± 0.02b	0.16 ± 0.06a	0.12 ± 0.06a
	Severely infested trees	0.08 ± 0.04a	−0.21 ± 0.04a	0.26 ± 0.06a	0.32 ± 0.06b

showing a clear narrow ring in 1953, thin transversal sections (15–20 μm thick) were obtained using a sliding microtome (Anglia Scientific AS 2000, UK). Sections included the 1953 ring and two 5-year reference periods, previous (1948–1952) and subsequent (1954–1958) to the outbreak. Sections were mounted on glass slides, stained with safranin (0.5% in distilled water), and fixed with Eukitt®. Images of sections were first taken at 40–100x magnification with a digital camera mounted on a light microscope (Olympus BH2), and then stitched with the ICE software (Microsoft©).

Images were analyzed for xylem measurement using the software ImageJ v.1.40 (Schneider et al., 2012). Lumen radial diameter (LD) and the double cell wall thickness (CWT) were measured for each tracheid along five radial rows for the 1948–1958 period using the 'plot profile' function. This function allowed discriminating between tracheid walls and lumen according to the variation of pixels intensity along a line passing through a tracheid radial file. To compare patterns of cell dimensions between and within rings, the number of cells was standardized to 20 by using the tracheidogram method (Vaganov et al., 2006) with the 'tgram' package (DeSoto et al., 2011) in the R statistical environment (R Core Team, 2017).

Table 3

Selected linear mixed-effects models of Scots pine radial growth (basal area increment) based on the degree of mistletoe infestation. Abbreviations of monthly climate variables: Pr, precipitation; TMn, mean minimum temperature; TMx, mean maximum temperature. Months are indicated by numbers and those corresponding to the year prior to growth are followed by "p". The Akaike weight (w_i) is the relative probability quantifying if the selected model is the best one.

Site	Infestation class	w_i	Parameters	Estimate (standard error)	*p*
Noguera (wet site)	Non-infested trees	0.62	Intercept	0.252 (1.082)	0.379
			Pr1	0.004 (0.001)	0.003
			Pr6	0.006 (0.002)	0.002
			TMn2	0.135 (0.064)	0.035
			TMn9	0.110 (0.042)	0.008
			TMx7	−0.105 (0.036)	0.031
	Severely infested trees	0.47	Intercept	0.282 (1.103)	0.798
			Pr12p	0.003 (0.002)	0.049
			Pr1	0.005 (0.002)	0.002
			Pr2	0.006 (0.001)	0.001
			Pr6	0.007 (0.002)	0.001
			TMn2	0.156 (0.020)	0.003
			TMx6	−0.073 (0.032)	0.024
			TMx7	−0.178 (0.045)	0.001
Manzanera (dry site)	Non-infested trees	0.26	Intercept	1.150 (2.532)	0.001
			Pr1	0.008 (0.003)	0.008
			Pr2	0.010 (0.005)	0.006
			Pr5	0.003 (0.001)	0.065
			Pr6	0.018 (0.002)	0.001
			TMn2	0.202 (0.086)	0.010
			TMx5	−0.191 (0.077)	0.013
			TMx7	−0.531 (0.129)	0.001
	Severely infested trees	0.33	Intercept	1.970 (2.727)	0.001
			Pr12p	0.014 (0.004)	0.001
			Pr1	0.005 (0.003)	0.048
			Pr6	0.016 (0.003)	0.001
			TMn2	0.466 (0.128)	0.005
			TMx5	−0.255 (0.075)	0.001
			TMx7	−0.581 (0.149)	0.001

2.7. Carbon isotope discrimination

We used the discrimination of C isotopes in wood ($^{13}C/^{12}C$, $\delta^{13}C$) to infer changes in intrinsic water-use efficiency (*WUEi*) (McCarroll and Loader, 2004). We selected basal samples of stem wood for two 21-year periods when mistletoe infestation was assumed to be low (1959–1979) and high (1991–2011), respectively. We took sections of five severely infested trees per site, which were sanded and visually cross-dated as the cores. Since logging at the dry site (Manzanera) was not allowed, sections were obtained from a nearby site with similar climate conditions (Alcalá de la Selva, 40° 22′ N, 0° 42′ W, 1660 m) previously sampled (Sangüesa-Barreda et al., 2013).

We obtained two 21-ring pools per site (corresponding to the 1959–1979 and 1991–2011 periods) by mixing the samples of five severely infested trees sampled at each site and showing similar size and age. Wood samples were homogenized to a fine powder using a mill (Retsch MM301, Haan, Germany). Wood aliquots (0.001 g) were weighed on a microbalance (AX205 Mettler Toledo, OH, USA) into tin foil capsules and analyzed with a Finnigan MAT Delta C isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., MA, USA). Isotope analyses were conducted at the Stable Isotope Facility of the University of California at Davis (USA). Stable isotope ratios were expressed as per mil deviations using the δ notation relative to Vienna Pee Dee Belemnite (VPDB). The standard deviation for repeated analyses was better than 0.2‰.

We estimated *WUEi* values from tree-ring wood C isotopes using the formulae proposed by Farquhar et al. (1982), and Francey and Farquhar (1982):

$$WUEi = Ca\ [1-(Ci/Ca)]\ 0.625, \tag{3}$$

where *Ca* is the ambient air CO_2 concentration, *Ci* is the CO_2 concentration in the sub-stomatal cavity of leaves, and 0.625 is the relation among the conductance of H_2O compared to the conductance of CO_2. The *Ci* was calculated as:

$$Ci = Ca\ (\delta^{13}Cw - \delta^{13}Ca + 1)/(b - a)] \tag{4}$$

where $\delta^{13}Cw$ and $\delta^{13}Ca$ are the C isotope discrimination values in the wood and in the atmosphere, respectively, *b* is the Rubisco enzymatic biologic fractionation (+27.0‰), and *a* is the diffusion fractionation across the boundary layer and the stomata (+4.4‰). Warm and dry conditions trigger stomatal closure, and thus the $\delta^{13}C$ becomes less negative and *WUEi* increases (Francey and Farquhar, 1982).

2.8. Statistical analyses

Trends in annual climate variables were assessed using the non-parametric Kendall tau (τ) statistic. To compare tree features between infested and non-infested trees (size, BAI) we used Mann-Whitney tests.

To detect differences in climate-growth relationships between non-infested and severely infested trees we used the following linear mixed-effects model:

$$Y_i = X_i\beta + Z_ib_i + \varepsilon_i \tag{5}$$

where Y_i is the response variable (annual, standardized BAI for a given tree), β is the vector of fixed effects (climate variables), b_i is the vector of random effects (tree Dbh and age at 1.3 m), X_i and Z_i are, respectively, fixed- and random-effects regressor matrices, and ε_i is the within group error vector. BAI models were fitted separately to severely infested and non-infested trees of the two study sites considering the common period 1930–2011. BAI of the previous year was introduced into the model for dealing with the first-order temporal autocorrelation of this variable, while tree Dbh and age accounted for size and age effects, respectively. Residuals of the models were checked for normality, homoscedasticity, and autocorrelation. The random effects and the covariance parameters were estimated using the restricted maximum likelihood method, and we also evaluated the existence of multicollinearity among fixed effects by calculating the variance inflation factor (Zuur et al., 2009). An information-theoretic approach was used for multi-model selection (Burnham and Anderson, 2002) by minimizing the Akaike Information Criterion corrected for small sample sizes (AICc). We selected the model showing the lowest AICc, and calculated its Akaike weight (w_i, relative probability quantifying if the selected model is the best one). Linear mixed-effects models were implemented in the *lme* function of the *nlme* library in the R software (R Development Core Team, 2017).

In the case of wood-anatomy data, we conducted a disturbance analysis using the Breaks for Additive Season and Trend algorithm, which detects changes in time series by calculating the deviation of the observed value with respect to the expected value estimated by the model (Cai and Liu, 2015). The ordinary least squares residuals-based moving sum test was used to test for whether one or more significant ($p < 0.05$) breakpoints occurred along the wood-anatomy time series (Zeileis, 2005). The *bfast* (Verbesselt et al., 2012) and *strucchange* (Zeileis et al., 2002) packages of the R software (R Development Core Team, 2017) were used in these analyses. Lastly, we used *t* tests and ANOVAs to check if there were significant differences in both tracheid traits (LD, CWT) between the outbreak (1953), the previous (1948–1952), and subsequent 5-year periods (1954–1958).

3. Results

3.1. Climate trends, tree size and recent growth patterns

Climate is warming in both study sites, whereas annual precipitation did not show any significant trend (Fig. 2). Severely infested trees

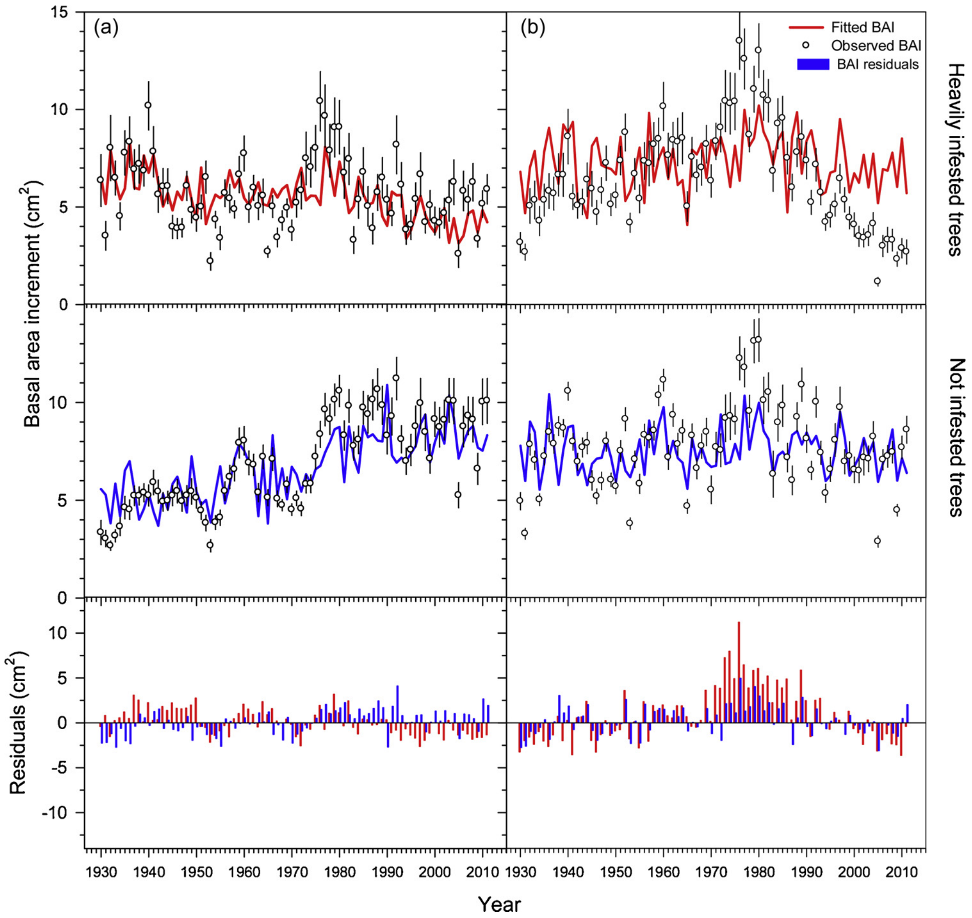

Fig. 4. Observed (symbols) and modeled (lines) values of radial growth (BAI, basal area increment) of Scots pines in the Noguera (a, wet site), and Manzanera (b, dry site) forests considering trees severely infested by mistletoe (red lines and bars), and non-infested trees (blue lines and bars). The lowermost plots show the residuals (difference between observed and fitted BAI values). See models' parameters in Table 3.

were taller, and showed lower growth rates during the last ten years than non-infested trees in both study sites (Table 1).

3.2. Growth responses to the insect outbreak and to mistletoe infestation

In both study sites, and regardless the level of mistletoe infestation, BAI showed a sharp reduction in 1953 corresponding to the *L. monacha* outbreak (Fig. 3). In Manzanera, BAI also decreased in 1924, whereas in Noguera the growth loss corresponding to the 1920s outbreak occurred in 1921-1924. Neither the long- (BAI slope of the 1953–1973 period) nor the short-term growth recovery (resilience) after 1953 differed between severely infested and non-infested trees in both sites, albeit both the slope and the resilience were higher when considering all trees in the dry (slope = 0.05 cm^2 yr^{-1}, RGD = 0.21) than in the wet (slope = 0.02 cm^2 yr^{-1}, RGD = 0.47) site (Table 2).

The BAI of non-infested trees was significantly ($p < 0.05$) higher than BAI of severely infested trees during the 1978–2011 and 1991–2011 periods in the wet (Noguera) and dry (Manzanera) sites, respectively (Fig. 3). In the wet site, severely infested trees grew more than non-infested trees prior to the 1950s outbreak. In the dry site, non-infested trees showed higher growth rates than infested trees, particularly before the 1920s outbreaks.

In both sites, the BAI slope of the 1991–2011 period, when mistletoe infestation was intense, was significantly lower in severely infested than in non-infested trees (Table 2). The lowest slope (-0.21 cm^2 yr^{-1}) was observed in the severely infested trees from the dry site. The post-2005 resilience also decreased in the severely infested trees as compared with their non-infested conspecifics, reaching the lowest values (highest RGD) in the wet site.

3.3. Climate-growth relationships as a function of mistletoe infestation

The growth (BAI) of severely infested trees was more sensitive to warm and dry spring-summer conditions as compared to non-infested trees (Table 3). These climatic conditions were more associated to growth reduction in severely infested than in non-infested trees, and these differences were more notable in the wet than in the dry site. Wet conditions during the prior winter and current spring also enhanced radial growth, but differences between infestation classes were not observed in this case.

The BAI models showed better fits in the wet than in the dry site (Table 3, Fig. 4), and the highest Akaike weight was found for non-infested trees. Negative BAI residuals in the severely infested trees suggested a growth loss related to mistletoe infestation, and not explained, by climate from the 1990s onwards (Fig. 4). In the dry site, positive BAI residuals in the 1970s indicated a growth increase not captured by the climate models in both tree classes.

3.4. Changes in wood anatomy associated to the *Lymantria monacha* outbreak

The tracheid lumen diameter (LD) in 1953 was significantly different than in the prior (1948–1952), and subsequent (1954–1958) periods ($F = 5.76$, $p = 0.0055$; see Fig. 5).

Lumen diameter in the outbreak year (1953) was significantly smaller than in the previous ($t = -3.39$, $p = 0.003$), and subsequent ($t = -2.95$, $p = 0.012$) 5-year periods, whereas the pre- and post-outbreak periods did not significantly differ ($t = 0.76$, $p = 0.721$). Lumen diameter recovered two years after the outbreak. Cell-wall thickness (CWT) values did not differ between 1953 and the other compared periods.

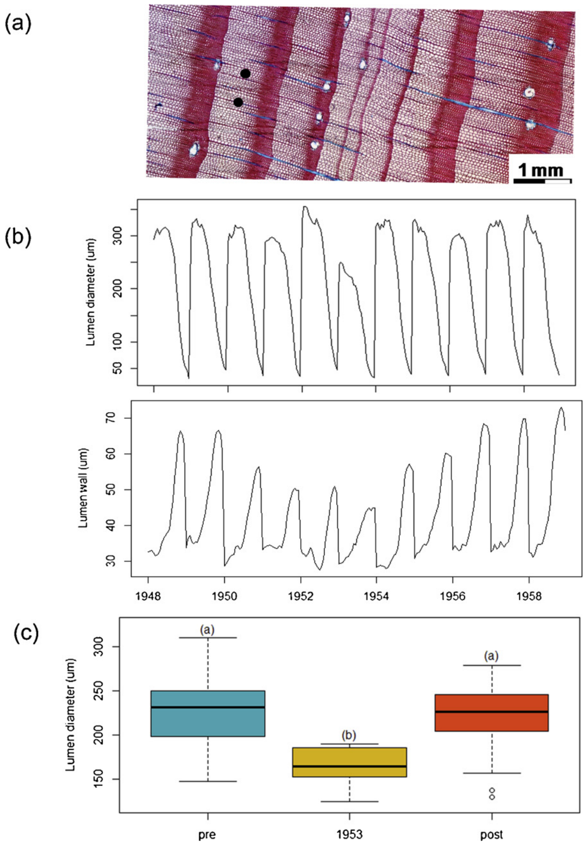

Fig. 5. Changes in wood anatomy related to the 1953 *Lymantria monacha* outbreak. Image showing the narrow 1953 ring (a) in a tree from the Noguera (wet) site (the two dots indicate the 1950 ring). Series showing the changes in lumen diameter and cell-wall thickness for the period 1948–1958 (b), and boxplots of lumen diameter comparing the 1953 lumen diameter with the values of the pre- (1948–1952) and post-outbreak (1954–1958) periods (c). Different letters indicate significant ($p < 0.05$) differences according to one-factor ANOVAs. The values of lumen diameter and cell-wall thickness are multiplied by 10.

Table 4
Values of intrinsic water use efficiency (*WUEi*) measured in basal samples of stem wood for two 21-year periods when mistletoe infestation was assumed to be low (1959–1979) and high (1991–2011), respectively. Data correspond to wet (Noguera) and dry (Alcalá de la Selva) Scots pine forest sites. Values are means ± SE. Different letters indicate significant differences between sites and within each period ($p < 0.05$, Mann-Whitney tests).

Periods	*WUEi* (μmol mol^{-1})	
	Wet site	Dry site
Pre-infestation period (1959-1975)	82.5 ± 1.0a	84.5 ± 1.3a
Infestation period (1991-2011)	95.7 ± 1.8a	104.8 ± 2.0b

3.5. Mistletoe infestation and water-use efficiency

The *WUEi* increased during the infestation period as compared with the pre-infestation period, but differences between periods were only significant in the dry site (Table 4).

4. Discussion

As hypothesized, mistletoe infestation caused a long-term growth reduction and an increase in *WUEi*, particularly in the dry site, whereas the 1950s *L. monacha* outbreak caused a short-term growth reduction, and a decrease in the number and lumen diameter of tracheids.

The higher growth (prior to mistletoe infestation) and height of severely infested vs. non-infested trees agrees with previous studies (Sangüesa-Barreda et al., 2012) Such patterns can be explained because mistletoe berries are dispersed by birds, which may preferentially perch on the tallest trees with biggest branches, and more illuminated canopies, which enhance the mistletoe establishment (Sangüesa-Barreda et al., 2018). Nevertheless, in the dry site currently infested trees grew less in the past than their non-infested counterparts, which could suggest the effects of past infestations or that they were subjected to more intense tree-to-tree competition. However, we did not observe growth releases, which could indicate past thinning activities in the study stands, excepting the growth increase during the 1970s in the dry site, which was not fully accounted by the climate model.

In other Scots pine dry sites (annual precipitation between 400 and 450 mm), the difference in growth between trees with or without mistletoe infestation occurred ca. 10 years prior to tree sampling, while the *WUEi* was significantly lower on severely infested trees only for the last 5 years (Sangüesa-Barreda et al., 2013). Such recent difference in *WUEi* translated into a. ca. 7% lower *WUEi* in the case of severely infested trees. In this study, the period with differential growth lasted longer, 34 (1978–2011) and 21 years (1991–2011) in the wet and dry sites, respectively, leading to growth reductions of 65% and 54%. The longer periods of differential growth could be related to the more mesic conditions of the two study sites as compared to those studied by Sangüesa-Barreda et al. (2013), but the relative radial-growth reduction was higher in the wet than in the dry site. Such result indicates that the infestation assessments and consequences on wood production should be improved since it might be the case that infestation was higher or impacted more trees in the wet (Noguera) than in the dry (Manzanera) site. Possibly, wet climate conditions allow trees hosting bigger mistletoe populations with higher density, but this idea should be further investigated. The characterized periods of differential growth are in agreement with the maximum age of mistletoe individuals (ca. 30 yrs.) in severely infested trees (Sangüesa-Barreda et al., 2013) suggesting a long infestation period in both study sites. Lastly, to reveal mistletoe effects on forest productivity we suggest focusing on severely infested trees, which show significant reductions in wood production regardless site conditions (Stanton, 2006).

The prolonged deviation in BAI between non-infested and severely infested trees also agrees with the relative increases of *WUEi* from the pre-infestation to the infestation periods, which were +16% (+0.46% yr^{-1}) and +24% (+0.69% yr^{-1}) in the wet and dry sites, respectively. The higher growth loss and lower *WUEi* increment in the wet site associated with the severe mistletoe infestation suggest that fast-growing trees at similar favorable locations may be also very vulnerable to mistletoe. Mistletoe infestation reduced growth, and also slowed down the increase in *WUEi* (Sangüesa-Barreda et al., 2013). However, the mistletoe effects on *WUEi* were less pronounced in wet than in dry sites, as expected. This indicates that Scots pine trees from wet sites will show higher resilience and resistance to long-term mistletoe infestation. Such finding is relevant because warming-related drought stress could affect more mesic Mediterranean forests (e.g. high-elevation pine stands) in the future contributing to a reduction in their productivity (Camarero et al., 2015), and uncoupling their growth and *WUEi* trends (Shestakova et al., 2017).

The short-term growth resilience after the 1953 outbreak was higher in the dry site (RGD = 0.21) than in the wet site (RGD = 0.47), but did not differ between mistletoe infestation classes. Recent mistletoe infestation affected the short-term growth resilience after the 2005 drought and reduced it 49% and 38% in the wet and dry sites, respectively. During the infestation period, the long-term growth drop was the highest in the severely infested trees from the dry site indicating synergistic effects of drought and mistletoe, which enhances water loss through leaves (Zweifel et al., 2012), on radial growth and wood production. The short-term resilience after the 2005 drought reached minimum values in the severely infested trees from the wet site suggesting a higher sensitivity of productive sites to the combined effects of long-term mistletoe infestation and drought.

This short-term sensitivity in the wet site agrees with the climate models, which accounted for more BAI variability there than in the dry site. Warm and dry spring-summer conditions constrained growth, in agreement with previous research on Mediterranean pinewoods (Camarero et al., 2015), particularly in severely infested trees from the wet site. These climatic conditions are associated to increased evapotranspiration and water shortage, and Scots pine trees from dry sites could be more acclimated to those constraints than trees from more wet sites (Voltas et al., 2013). Mistletoe infestation would amplify the different impact of climate conditions by exacerbating transpiration and drought stress on a typically isohydric species with tight control of stomata conductance as Scots pine (Rigling et al., 2010). In ponderosa pine (*Pinus ponderosa*), mistletoe-infected trees were also more sensitive to drought than uninfected trees (Stanton, 2007).

We were able to link a past *L. monacha* outbreak, which was described in the 1950s (Rupérez, 1958), with a severe growth reduction and a significant decrease in the tracheid number and lumen diameter during 1953 both in the wet and dry sites, but with a higher impact on growth in the wet site where the post-outbreak resilience was the lowest. Previous studies detected similar growth reductions lasting 1–3 years and linked to *Lymantria* spp. outbreaks and defoliations (Muzika and Liebhold, 1999; Kochanowski and Bednarz, 2007), which occurred during dry years as in this case, albeit they did not analyze changes in wood anatomy. In the wet site, the outbreak caused a 33% relative reduction of lumen diameter, whereas growth was reduced by ca. 50% reflecting the importance of the loss in hydraulic conductivity caused by the insect defoliation. However, the growth reduction was much more important in severely infested trees (–80%) than in non-infested trees (–20%). The 1953 outbreak reduced more growth of fast-growing, severely infested trees, which had bigger crowns, than in slow-growing, non-infested trees from the wet site. The growth rates after the 1950s outbreak, from 1955 to 1970, were similar between the two infestation classes. The 1920s outbreak caused severe growth reductions in different years, 1921 and 1924 in the wet and dry sites, and its effects were possibly not as important since growth rates of the two infestation classes remained different afterwards. Finally, the statistical technique used to detect the sharp reduction in lumen diameter could be applied to detect and recognize other disturbances affecting wood anatomy (droughts, frosts, etc.), which should be corroborated with historical records.

To conclude, mistletoe infestation caused a long-term growth reduction, and an increase in *WUEi*, particularly in the dry site, and negatively impacted the short-term, post-drought growth resilience in the wet site. The *L. monacha* outbreak caused a short-term growth reduction, particularly in severely infested trees from the wet site, and a decrease in the lumen diameter of tracheids. Warmer and drier conditions during the growing season negatively impacted growth of severely infested trees, especially in the wet site. This study reveals: (i) the relevance of past growth legacies due to biotic stressors (outbreak), which can restrict the responses of trees to recent stressors (mistletoe, climate warming and drought), and (ii) the usefulness of tree-ring and wood-anatomy data to reconstruct the long- and short-term effects of stressors on growth.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.dendro.2019.05.002.

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