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## Research paper

# Mistletoe effects on Scots pine decline following drought events: insights from within-tree spatial patterns, growth and carbohydrates

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Forest decline has been attributed to the interaction of several stressors including biotic factors such as mistletoes and climate-induced drought stress. However, few data exist on how mistletoes are spatially arranged within trees and how this spatial pattern is related to changes in radial growth, responses to drought stress and carbon use. We used dendrochronology to quantify how mistletoe (*Viscum album* L.) infestation and drought stress affected long-term growth patterns in *Pinus sylvestris* L. at different heights. Basal area increment (BAI) trends and comparisons between trees of three different infestation degrees (without mistletoe, ID1; moderately infested trees, ID2; and severely infested trees, ID3) were performed using linear mixed-effects models. To identify the main climatic drivers of tree growth tree-ring widths were converted into indexed chronologies and related to climate data using correlation functions. We performed spatial analyses of the 3D distribution of mistletoe individuals and their ages within the crowns of three severely infested pines to describe their patterns. Lastly, we quantified carbohydrate and nitrogen concentrations in needles and sapwood of branches from severely infested trees and from trees without mistletoe. Mistletoe individuals formed strongly clustered groups of similar age within tree crowns and their age increased towards the crown apex. Mistletoe infestation negatively impacted growth but this effect was stronger near the tree apex, causing an average loss of 64% in BAI than in the rest of sampled heights (loss of ~51% in BAI computed at 1.3 m or near the tree base). We found that BAI of severely infested trees and moderately or non-infested trees diverged since 2001 and such divergence was magnified by drought. Infested trees had lower concentrations of soluble sugars in their needles than non-infested branches. We conclude that mistletoe infestation causes growth decline and increases the sensitivity of trees to drought stress.

**Keywords:** basal area increment, drought, *Pinus sylvestris*, point pattern analysis, *Viscum album*.

## Introduction

Forest decline has been detected worldwide being linked to multiple interacting factors including biotic agents and drought-induced stress (Allen et al. 2010). Following the Manion (1991) decline model, drought-stressed trees may be negatively affected or even finally be killed off by secondary biotic agents such as mistletoes, insects and pathogenic fungi (McDowell et al. 2011). Interactions among decline drivers may be complex

because climate change may also alter tree–organism relationships in drought-prone ecosystems with important implications for tree growth and survival (Ayes and Lombardero 2000). For instance, warming-induced drought stress may make host trees reinforced more vulnerable to the negative effects of hemiparasitic plants as mistletoes. However, few detailed data exist on how mistletoe infestation and drought affect tree growth through time and whether these two stress factors

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have a combined negative effect. Furthermore, a careful examination of radial growth patterns within trees, i.e., along their main stem, may allow assessing the effects of mistletoe infestation on wood production, which is a reliable proxy for carbon acquisition and likelihood of tree death (Ogle et al. 2000).

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Mistletoes are perennial and aerial hemiparasitic plants of trees and take from their hosts water, photosynthates and mineral nutrients, thus increasing the drought stress and compromising the carbon balance of the host tree, particularly in areas with pronounced water deficit (Glatzel and Geils 2009). The main hosts of European pine mistletoe (*Viscum album* ssp. *austriacum* L.) are Scots pines (*Pinus sylvestris* L.) (Zuber 2004). Previous studies indicate that pine mistletoe can adversely affect long-term tree growth and vigour in drought-prone areas (Dobbertin et al. 2005). For instance, in dry inner valleys of the Swiss Alps, Scots pines infested by mistletoe showed mortality rates to be more than twice that of non-infested trees, and most defoliated trees were among the most vulnerable individuals to stress imposed by mistletoe on its host tree (Dobbertin and Rigling 2006). Rigling et al. (2010) suggested that pine mistletoe increases the risk of drought-induced mortality of its host making trees more susceptible to drought stress when growing in a xeric environment due to the enhanced needle loss. Such mistletoe-related defoliation of tree crowns was also suggested as a decline driver of trees affected by dwarf mistletoes in Northern American forests (Geils and Hawksworth 2002).

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To the best of our knowledge, there are no detailed within-crown assessments of mistletoe presence in severely infested trees, considering the position and age of mistletoe individuals through the crown and the stem, although these data may provide a description of the invasion pattern and allow inferring how the mistletoe infestation occurs and how it affects tree performance (Vallauri 1998). In addition, such a descriptive approach of the infestation process should be combined with a quantification of the effects of mistletoe on carbon and nutrients storage and wood formation. This would allow disentangling the relative effects of drought stress and mistletoe on long-term growth trends of trees. To fill these research gaps, we performed 3D point pattern analyses of mistletoe individuals within tree crowns, used dendrochronology for assessing retrospectively long-term changes in radial growth at different heights along the main stem and analysed the concentration of non-structural carbohydrates (NSC) and nitrogen (N) in needles and sapwood from severely infested trees and trees without mistletoe to determine if mistletoe affects carbon and N content in branches. We aimed at: (i) describing and quantifying the spatial patterns of mistletoe individuals according to their age in severely infested host trees, (ii) assessing the radial growth trends at different heights as a function of mistletoe abundance and drought stress and (iii) determining if mistletoe presence affects the

concentration of NSC and N in sapwood and needles of infested branches.

## Materials and methods

### Study site

The study site is a Scots pine (*Pinus sylvestris* L.) forest located in Teruel, Eastern Spain (40°22'26"N, 0°40'14" W, 1585 m a.s.l.), i.e., near the southern distribution limit of the species (Figure 1). The mean density and basal area of the site are 600 trees ha<sup>-1</sup> and 19.4 m<sup>2</sup> ha<sup>-1</sup>, respectively. The site is located on an almost flat slope (0–5°). The climate in the study area is Mediterranean with continental influence. The mean annual temperature is 9.4 °C and the annual precipitation is 670 mm (see Table S1 and Figure S1 available as Supplementary Data at *Tree Physiology* Online).

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### Climate data

To obtain a robust regional climatic series, local data from four meteorological stations located from 10 up to 30 km from the study site were combined into a regional mean for the period 1954–2008 (see Table S1 available as Supplementary Data at *Tree Physiology* Online). To estimate the missing data for each station, and to combine them, we used the MET program from the Dendrochronology Program Library (Holmes 1994). For each station, monthly variables (mean temperature and total precipitation) were transformed into normalized standard deviations to give each station the same weight in calculating the average monthly values for each year.

We calculated a regional cumulative water deficit using a modified Thornthwaite water-budget procedure based on the mean temperature and total precipitation monthly data (Willmott et al. 1985). Soil water balance was modelled by estimating soil water withdrawal, recharge and surplus using

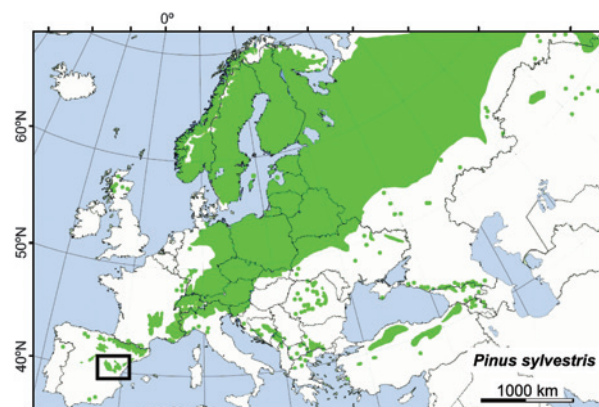


Figure 1. Distribution area of *Pinus sylvestris* in Europe (redrawn from distribution maps available in <http://www.euforgen.org/>) and study sites (square) in Teruel (Eastern Spain), near the southern distribution limit of the species.

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the AET calculator program developed by D.G. Gavin (<http://geography.uoregon.edu/envchange/pbl/software.html>). The soil water balance is modelled on a daily time step by estimating actual evapotranspiration (AE), recharge and surplus. Actual evapotranspiration is estimated from potential evapotranspiration (PE) and the percentage of the current soil, which is at the available water capacity (AWC). Potential evapotranspiration is estimated empirically from monthly mean temperature and day length. Actual evapotranspiration is computed by multiplying PE by a 'declining availability function', which models how the ratio AE/PE ratio drops to zero once soil water is lower than 30% of AWC. This function assumes that as soil water is drawn down during the summer, it is increasingly difficult to extract water due to soil cohesion. Available water capacity at the study sites was estimated as 120 mm based on soil texture data and assuming a mean soil depth of 40 cm. Soil water recharge will occur on days when snowmelt and/or precipitation exceed soil water withdrawal. When soil water is at AWC, any snowmelt and precipitation beyond that required by PE is considered surplus and is not available on following days. Daily snowmelt is empirically estimated from the temperature, the amount of accumulated snow and daily precipitation, which is based on monthly precipitation distributed evenly over the month. Based on that, severe drought events were defined as those years with the maximum cumulative water deficit values (1986, 1994 and 2005). We calculated the cumulative water deficit from January to June, when the Scots pine performs most of its radial growth (Gutiérrez 1989). Positive and negative values correspond to wet and dry conditions, respectively. The mean cumulative water deficit ( $\pm$ SE) for the period 1954–2008 was  $-21.7 \pm 21.2$  mm, whereas values for the selected dry years were as follows:  $-117.8$  mm for 1986,  $-165.4$  mm for 1994 and  $-122.3$  mm for 2005.

### Field sampling

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Sampling was done in September 2008. Trees with contrasting levels of mistletoe abundance were selected within a plot of surface equal to 0.5 ha randomly located in the studied forest. Mistletoe abundance was estimated using a modified three-class rating system based on the original scale established by Hawksworth (1977). The tree crown was divided into three similar vertical parts and each third was rated from 0 (absence of mistletoe) to 1 (presence of mistletoe) as a function of the abundance of mistletoe individuals (Figure 2). Then, the total mistletoe abundance or infestation degree of each tree was obtained summing the rates of each crown third. Mistletoe abundance ranged from 0 to 3 and all analyses were performed based on three classes of abundance or infestation degree (ID): trees without mistletoes (ID = 0, class ID1), moderately infested trees with mistletoe present in one or two thirds of the crown (ID = 1–2, class ID2) and severely infested

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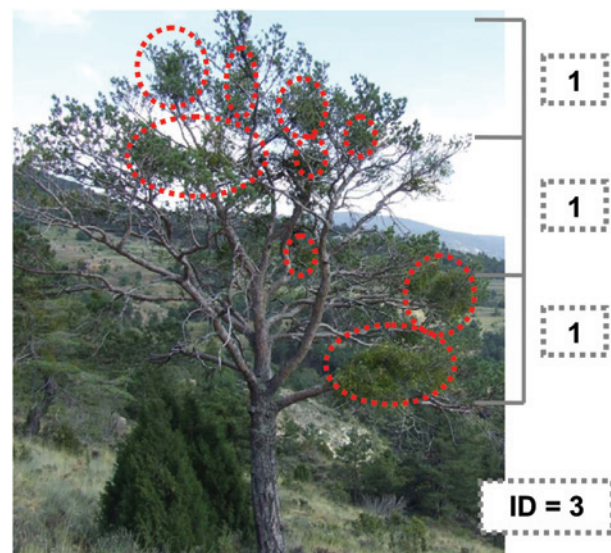


Figure 2. Example of rating of mistletoe infestation degree (ID). This tree was regarded as a severely infested tree because mistletoe individuals (delineated with dotted ellipses) were present in the three thirds of its crown (ID = 3, class ID3).

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trees with mistletoe present throughout the crown (ID = 3, class ID3) (see also Geils and Hawksworth 2002).

Trees were logged to take wood discs at three different heights per tree: apex (0.5–1 m from the tree main apex), 1.3 m (diameter at breast height, dbh) and base (0.5–1 m above the soil and the assumed shoot–root collar). Sapwood area was measured in the field in wood sections taken at 1.3 m. We checked these field visual estimates by applying bromocresol green stain on recently collected cores in the laboratory (Kutscha and Sachs 1962). We sampled 20 trees without mistletoe (ID1), 10 trees with moderate abundance of mistletoe (ID2) and 10 trees severely infested (ID3). We randomly selected and carefully sampled the crown of three trees severely infested (ID3) to describe the spatial pattern and age of mistletoe individuals in their canopies.

We measured for all trees their size (dbh; tree height; crown height and diameter—the latter was based on the average of two horizontal crown diameters measured along N–S and E–W directions) and per cent crown cover to estimate the needle density within the crown. Since estimates of per cent crown cover may vary among observers and places, we used as a reference a tree with the maximum amount of foliage at each site and this variable was always measured by the first author.

To describe the spatial patterns of mistletoe individuals located within the crown of severely infested trees we randomly selected three pines. These three logged and severely infested trees were placed on the floor along the north side of the trunks to allow comparison of the patterns of mistletoe distribution among them. Within each of these three trees we measured their relative coordinates ( $x$ ,  $y$ ,  $z$ ) and their basal

diameters and quantified mistletoe ages. Spatial coordinates were obtained measuring three distances from each mistletoe individual to: the main stem of the tree (coordinate  $x$ ), the tree base (coordinate  $y$ ) and an imaginary line pointing towards the north (coordinate  $z$ ). We acknowledge the fact that the smallest, and plausibly youngest, mistletoe individuals (e.g., 1–2 year old individuals) may not have been detected, but we carefully examined all living branches found in these three intensively sampled trees (Table 1). Mistletoe age was estimated counting the number of annual internodes along the oldest axis of the plant and adding 1 year following Zuber (2004). In the case of old mistletoe individuals (age > 15 years), whose basal internodes may be tightly packed, ages may also have been underestimated. Repeated estimations in middle-aged (age = 10–15 years) mistletoes ( $n = 50$ ) by the first and third authors yielded age errors equal to  $\pm 1$  year. Finally, we measured the basal diameter of all mistletoe individuals. Age and basal diameter were highly related and their associations followed logarithmic models of the form  $\text{age} = a + b \times \ln(\text{diameter})$  which reinforces our age estimations (see Table S2 and Figure S2 available as Supplementary Data at *Tree Physiology* Online).

### Dendrochronological methods

Wood samples were air dried and sanded with papers of progressively finer grain until tree rings were clearly visible. Two opposite radii were selected in each section and they were visually cross-dated. Then, tree-ring widths were measured to a resolution of 0.01 mm using a semiautomatic device (LINTAB, Frank Rinn, Heidelberg, Germany). The visual cross-dating was checked with the software COFECHA (Holmes 1983).

To quantify and compare growth trends between trees with low or high mistletoe infestation classes we transformed tree-ring width data into basal area increments (BAI) for the three heights considered and taking into account the three levels of mistletoe infestation (ID1, ID2 and ID3). Basal area increment removes variation in growth attributable to increasing stem circumference and captures changes in growth better than linear measures as tree-ring width (Biondi and Qeadan 2008). In further comparisons, we only considered the period 1995–2008 when the number of measured radii was constant and maximum. We also obtained relative BAI data dividing the mean BAI data for the mentioned period by the total wood area.

### Growth–climate associations

To compare the relationships between climate and growth at different heights, and as function of mistletoe infestation degree, we quantified growth–climate associations using correlation analyses. Individual tree chronologies were built from cross-dated ring-width series using the ARSTAN program by using a two-step detrending procedure (Cook 1985). The series were first fitted to negative or linear exponential functions, and then to a cubic smoothing spline with a 50% frequency response of 30 years to retain high-frequency variability. The detrended ring-width series were then standardized by transforming them into dimensionless indices by dividing the observed by the expected values given by the functions. Then, autoregressive modelling was performed on each series to obtain residual indexed series. Several descriptive statistics were calculated for the common interval 1974–2008 from the raw tree-ring widths (mean, SE and AC), and the residual chronologies (MSx,  $r_{bt}$ , EPS): SE is the standard error of tree-ring widths; AC is the first-order autocorrelation, a measure of the year-to-year growth similarity; MSx is the mean sensitivity which quantifies the year-to-year variability in width of successive rings;  $r_{bt}$  is the mean between-trees correlation which determines the similarity in growth among trees; and the EPS is the expressed population signal which is a parameter indicating the statistical quality of the chronology as compared with an infinitely replicated chronology (for details see Fritts 1976). The periods in which the growth series showed EPS values higher than 0.85 were considered as well replicated (Wigley et al. 1984).

Chronologies for the three sampled heights along the stem considering only trees without mistletoe (class ID1) were obtained by averaging the indexed values for each year using a bi-weight robust mean. Finally, growth–climate associations were quantified by calculating Pearson correlation coefficients between residual growth indices and monthly climatic variables (mean temperature, total precipitation) considering only trees without mistletoe for the period 1980–2008 because this was the common and well-replicated period for all wood samples. Correlations were calculated from the previous August up to October of the year of tree-ring formation, since climatic conditions during this 15-month period affect radial growth of *P. sylvestris* in sites with similar climatic conditions (Richter et al. 1991).

Table 1. Characteristics of the three intensively studied pines with severe mistletoe infestation.

Tree code	dbh (cm)	Height (m)	Crown height/diameter (m)	Crown cover (%)	Age (years)	No. mistletoe individuals
Pine 1	28.2	12.5	6.0/4.0	80	77	823
Pine 2	25.5	12.5	5.5/3.0	70	86	826
Pine 3	25.1	11.5	5.5/3.5	50	80	810

### Growth response to drought

Drought responsiveness was evaluated as the degree to which tree growth is affected by selected drought events. We defined drought sensitivity (DS) and drought recovery (DR) based on the relative changes in BAI for the three selected drought years (1986, 1994 and 2005) as follows (see Linares and Tiscar 2010):

$$DS = \left[ (BAI_D - BAI_{D-3}) / BAI_{D-3} \right] \times 100 \quad (1)$$

$$DR = \left[ (BAI_{D+3} - BAI_{D-3}) / BAI_{D-3} \right] \times 100 \quad (2)$$

where  $BAI_D$  is the BAI value for the drought year,  $BAI_{D-3}$  is the mean BAI of the 3 years before the drought year and  $BAI_{D+3}$  is the mean BAI of the 3 years after the drought year. To compare these two variables between dry years during the mistletoe infestation process and wet years before intense mistletoe infestation we also calculated DS and DR for a wet year (1983, mean cumulative water deficit = 77 mm) when the mistletoe infestation process was beginning or had not yet started.

### Superposed epoch analysis

To identify if growth showed punctual responses to drought events we used superposed epoch analysis which allows detecting growth drops from the mean indexed series in response to droughts as a function of mistletoe infestation and sampling height. We analysed departures for the 3 years before and after the selected drought years (1986, 1994, 2005). We tested the significance of mean tree growth response to these extremely dry years following Orwig and Abrams (1997). We randomly selected 10,000 sets of 7 years from each series and to estimate the confidence intervals ( $P < 0.05$ ) for the plotted growth departures (Haurwitz and Brier 1981, Lough and Fritts 1987).

### Spatial analyses

We used two types of spatial analyses: (i) point pattern analysis to find out if mistletoe individuals were clustered or randomly distributed within the trees' crowns and (ii) surface pattern analysis to describe the pattern of age of sampled mistletoe individuals. Firstly, we used a point pattern analysis for 3D datasets based on the nearest-neighbour  $G(t)$  function, which is the cumulative distribution function for the population of nearest neighbour distances and it is usually represented as a function of interpoint distances ( $t$ ) (Diggle 2003). This function is based on the distances between nearest neighbours which provide information about the pattern of points (Dixon 2002). To quantify how the 3D mistletoe patterns deviated from complete spatial randomness (CSR), the calculated  $G(t)$  functions were compared with a heterogeneous Poisson

process representing CSR and also with spatial null models. Values of  $G(t)$  higher (lower) than those estimated for the Poisson process correspond to clustered (regular) patterns (Møller and Waagepetersen 2003). To estimate the significance of the obtained  $G(t)$  values, they were compared with the upper and lower 5% intervals obtained after calculating  $G(t)$  values for 999 randomizations of the original data. Null models were calculated averaging the  $G(t)$  values obtained in the randomizations. The point pattern was analysed in the three intensively sampled trees for distances ( $t$ ) from 0 to 0.7 m at 0.05 m steps. To detect if mistletoes formed spatial aggregates based on their ages, these analyses were performed separately for mistletoe individuals younger and older than 7 years which is the mean age of reproductive maturity of these species, i.e., when individuals start flowering and females may produce fruits (Zuber 2004). The point pattern analyses were performed using the program Spatial Analysis 3D (Eglen et al. 2008) and assuming a volume equal to a rectangular prism comprising the entire tree canopy.

Secondly, to detect if mistletoe individuals formed clustered groups of similar ages we calculated spatial correlograms of mistletoe age based on the Moran  $I$  index and considering only the horizontal (distance to the tree main stem) and vertical (height) coordinates of mistletoe individuals. The Moran  $I$  index usually ranges between +1 and -1 with positive and negative values corresponding, respectively, to positive (e.g., neighbouring mistletoes have similar ages) and negative (e.g., neighbouring mistletoes have different ages) spatial autocorrelation (Fortin and Dale 2005). The distance at which the Moran indices approach the zero value is regarded as the mean size of patches with similar autocorrelation values. We calculated the Moran  $I$  index for 0.5-m steps from 0 up to 3.5 m and estimated the significance ( $P = 0.05$ ) of each index through randomization tests based on 999 permutations. Finally, the global significance ( $P'$ ) of each correlogram was calculated based on a Bonferroni correction considering the  $k$  distance classes analysed (in our case  $k = 7$ ) where  $P' = P/k$  (Legendre and Legendre 1998). Correlograms were calculated using the program Passage (Rosenberg and Anderson 2011).

### Chemical analyses

To assess the effect of mistletoe infestation on the concentration of NSC and N we sampled sapwood and current-year needles in branches from 10 trees without mistletoe individuals and from 10 trees severely infested (class ID3). Trees of both infestation classes did not significantly differ in dbh (infested,  $26.6 \pm 1.9$  cm; non-infested,  $24.9 \pm 1.7$  cm). Two 15-year-old branches were randomly selected from the southern-oriented and well-exposed upper third of the crown of each tree.

The concentrations of N, soluble sugars (SS), starch and NSC were measured in the needles and branch sapwood. Needle and wood samples were oven-dried and milled to a fine

powder (IKA MF10, IKA-Werke, Staufen, Denmark). Nitrogen concentrations were measured with an elemental analyser (Elementar varioMAX N/CN, Hanau, Germany). Soluble sugars were extracted with 80% (v/v) ethanol and concentrations were determined colorimetrically using the phenol–sulphuric method of Dubois et al. (1956) as modified by Buysse and Merckx (1993). Starch and complex sugars remaining in the undissolved pellet after ethanol extractions were enzymatically reduced to glucose and analysed as in Palacio et al. (2007). Non-structural carbohydrates measured after ethanol extraction are referred to as SS, carbohydrates measured after enzymatic digestion in glucose equivalents are referred to as starch, and the sum of SS and starch measured in glucose equivalents are referred to as total NSC. In the case of needles we also calculated the C : N ratios.

### Statistical analyses

We quantified the associations among variables using correlation analyses based on the Pearson ( $r$ ) and Spearman ( $r_s$ ) coefficients. To evaluate the effects of mistletoe infestation degree, time and their interaction on growth (log-transformed BAI) we used linear mixed-effects models for the period 1980–2008 considering both factors as fixed effects and trees as random effects (Littell et al. 2006). Models were fitted for the three sampling heights and considering tree diameter (dbh) as a covariate using SAS ver. 9.1.3 (SAS Institute, Cary, NC, USA).

## Results

### Mistletoe spatial patterns

Mistletoe individuals were strongly clustered at all analysed distances (see Figure S3 available as Supplementary Data at *Tree Physiology* Online). The clustering was more intense for mistletoes whose ages were lower than 7 years than for older ones. Overall, mistletoes were also more clustered in trees 2 and 3 than in tree 1 which agrees with the fact that the former trees had more branches forming horizontal strata than the latter one (see Figure S4 available as Supplementary Data at *Tree Physiology* Online). The mistletoe age also presented aggregated patterns within tree crowns since spatial Moran correlograms were all globally significant ( $P' < 0.05$ ) and corresponded to significant positive autocorrelations up to 1 m (pines 2 and 3) or up to 3 m (pine 1; Figure 3). Mistletoe age and basal diameter increased towards the crown apex (see Table S3 available as Supplementary Data at *Tree Physiology* Online).

### Mistletoe and drought effects on growth: roles of sampling height and infestation degree

Trees without mistletoe showed significantly (Mann–Whitney  $U$  test,  $U = 3.5–19.0$ ,  $P < 0.001$ ) higher crown cover values

( $95.5 \pm 2.0\%$ ) than moderately ( $80.6 \pm 3.8\%$ ) and severely infested trees ( $57.1 \pm 4.7\%$ ), and moderately infested trees also presented higher crown cover values than severely infested ones ( $U = 10.0$ ,  $P = 0.002$ ). The structural and growth variables most negatively related to mistletoe infestation degree were crown cover and BAI, both in absolute and in relative terms, of apical wood samples (Table 2). As expected, BAI was tightly linked to dbh, crown size (height and diameter) and crown cover.

The linear mixed-effects models showed highly significant and negative effects ( $P < 0.001$ ) of the infestation degree on BAI at all heights analysed, this effect being strongest in the apical samples ( $F = 19.83$ ) and weakest in the 1.3-m samples ( $F = 9.33$ ). The rest of the variables (time, interaction time  $\times$  ID and dbh) also presented significant effects on BAI (results not shown).

The BAI series showed how the trees without mistletoe (ID1 class) show higher increment than moderately (ID2 class) and severely (class ID3) infested trees but such divergence was apparent only since the early 2000s (Figure 4). Basal area increment differed significantly among infestation classes for the period 2000–08 (Tukey's honestly significant difference test,  $F = 13.12$ ,  $P = 0.0004$ ). In agreement with the linear mixed-effects models, the divergence of growth among trees with different infestation degrees was higher in apical wood samples than in the others. Finally, all trees showed growth reductions in response to droughts but water deficit increased the growth divergence among infestation classes, particularly in the last few decades (i.e., after the 2005 drought) and in apical samples. During the last 3 years, trees without mistletoe showed BAI values twice (or more in the apical samples) those observed in severely infested trees. However, after the previous two extreme droughts (1986, 1994) such divergences were not observed and BAI did not significantly differ among infestation classes (Figure 4).

Trees showed increasing drought sensitivity and decreasing drought recovery values as they were more infested by mistletoe and in recent droughts (e.g., 2005) than in previous (e.g., 1994) ones (Table 3). Drought sensitivity reached the lowest values (i.e., the highest relative BAI reduction) in the apical wood samples of severely infested trees (class ID3) and in response to the late 2005 drought. Accordingly, growth departures reached the minimum values the year of severe drought occurrence, and they were minimum ( $\sim 40\%$  of growth reduction) and significantly differed from simulated data in apical samples of severely infested trees (Figure 5).

Lastly, the statistics of the ring-width series (Table 4) indicated that the serial correlation in growth (AC, first-order autocorrelation) and the year-to-year growth similarity (MSx, mean sensitivity) were the lowest and the highest, respectively, in apical samples. We found that the growth variance explained by climate was much higher in apical (55%) samples than in

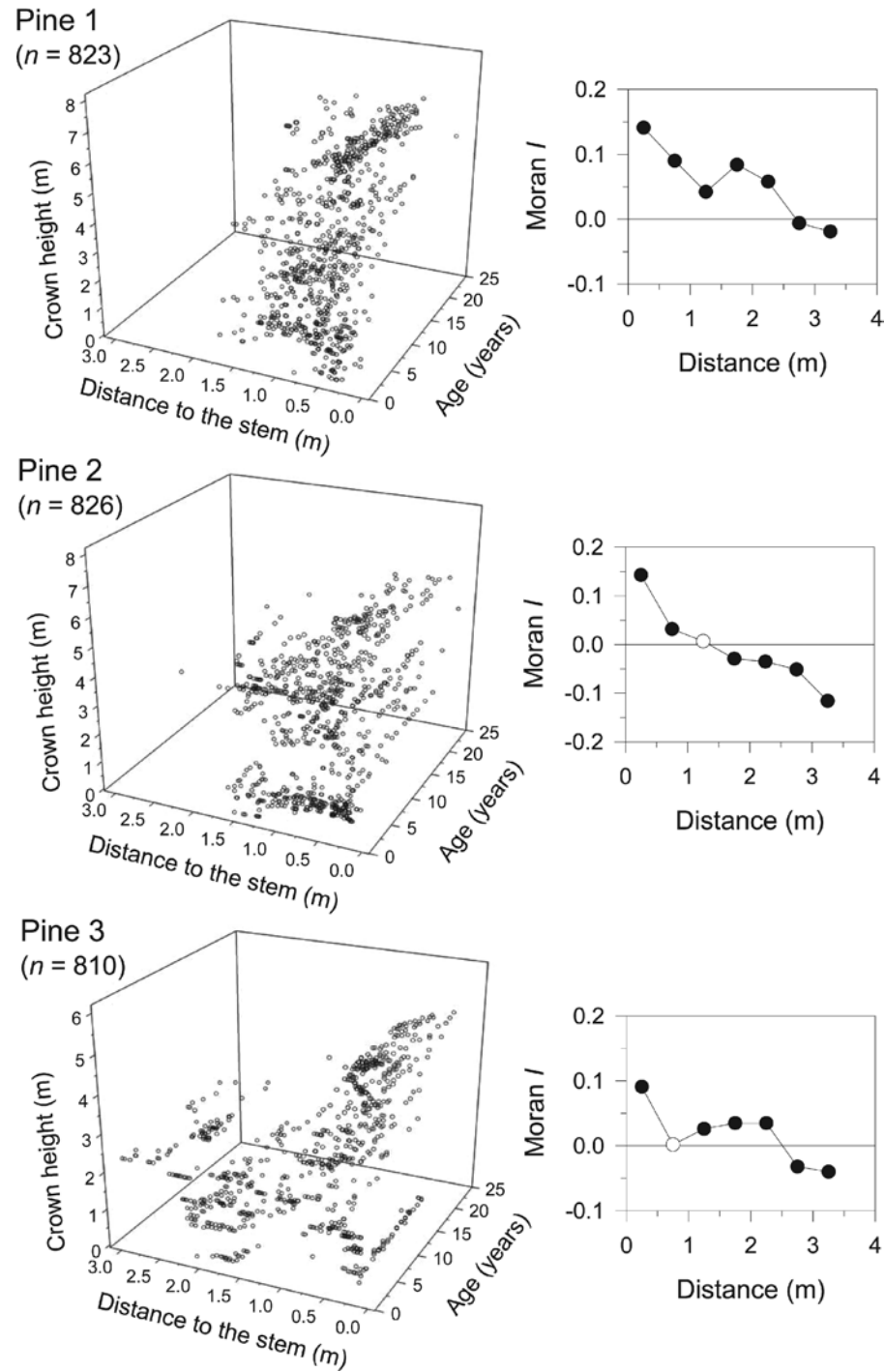


Figure 3. Spatial patterns of mistletoe age and related Moran spatial correlograms in the three intensively sampled pines. The aged mistletoe individuals located within each tree are indicated (see also Table 1). The spatial coordinates of mistletoes refer to their position within the crowns based on the distance to the main stem and the height along the crown. In the correlograms the filled symbols indicate significant ( $P < 0.05$ ) Moran  $I$  indices.

dbh (34%) or basal (28%) samples (Table 4). However, we detected similar responses of Scots pine growth to climate at the three analysed heights (see Figure S5 available as Supplementary Data at *Tree Physiology* Online). Stem growth was enhanced by April temperatures and by May and August precipitation, whereas warm temperatures in the previous

October and the current July were linked to narrow tree rings (see Figure S5 available as Supplementary Data at *Tree Physiology* Online). When we performed correlation analyses at the tree level between spring (March, April and May) precipitation and ring-width indices at the three sampled heights, only apical samples presented significantly ( $P < 0.05$ ) different

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Table 2. Relationships (Spearman's correlation coefficient) calculated between mistletoe infestation degree (ID) and tree variables considering the three sampled heights ( $n = 40$  trees). The upper, middle and lower lines within each cell correspond to wood samples taken near the apex, at 1.3 m (dbh) and near the base, respectively, whereas cells with a single value correspond to dbh samples. \*\*Significant ( $P \leq 0.05$ ) values are in bold.

	ID	BAI <sub>1995–2008</sub> (cm <sup>2</sup> year <sup>-1</sup> )	dbh (cm)	Height (m)	Crown height (m)	Crown diameter (m)	Crown cover (%)	BAI <sub>1995–2008</sub> (cm <sup>2</sup> year <sup>-1</sup> )
BAI <sub>1995–2008</sub> (cm <sup>2</sup> year <sup>-1</sup> )	<b>-0.51</b>							
	-0.18							
	-0.28							
dbh (cm)	0.07	0.28						
		<b>0.68</b>						
		<b>0.58</b>						
Height (m)	0.08	-0.18	0.13					
		0.17						
		0.02						
Crown height (m)	-0.17	<b>0.45</b>	0.29	0.07				
		<b>0.40</b>						
		0.26						
Crown diameter (m)	-0.05	<b>0.56</b>	<b>0.57**</b>	-0.10	<b>0.67</b>			
		<b>0.54</b>						
		<b>0.43</b>						
Crown cover (%)	<b>-0.75</b>	<b>0.65</b>	0.29	-0.06	<b>0.38</b>	<b>0.35</b>		
		<b>0.56</b>						
		<b>0.62</b>						
Sapwood area (%)	0.24	0.22	0.12	0.20	0.11	0.27	-0.18	-0.18
BAI <sub>1995–2008</sub> /basal area (year <sup>-1</sup> )	<b>-0.57</b>	<b>0.91</b>	-0.09	-0.19	<b>0.36</b>	0.31	<b>0.60</b>	0.20
	-0.25	<b>0.89</b>	<b>0.34</b>	0.17	<b>0.37</b>	<b>0.40</b>	<b>0.56</b>	
	<b>-0.34</b>	<b>0.91</b>	0.26	-0.06	0.21	0.30	<b>0.62</b>	

growth–climate correlations among infestation classes. The growth responses to spring rainfall were highest in the severely infested trees (ID3,  $0.47 \pm 0.03$ ), followed by moderately infested trees (ID2,  $0.38 \pm 0.03$ ) and lowest in trees without mistletoe (ID1,  $0.29 \pm 0.03$ ).

### Nitrogen and carbohydrate concentrations

The concentrations of sapwood carbohydrates (SS, starch and NSC) and needle N did not differ significantly when comparing branches from highly infested trees and those from trees without mistletoe (Table 5). Only needles from highly infested branches had lower concentrations of SS ( $P < 0.05$ ), starch ( $P = 0.08$ ) and NSC ( $P = 0.09$ ) than those from branches of trees without mistletoe (Table 5). Infested trees with higher needle loss showed higher N concentrations, and hence lower C:N ratios in their needles, than non-infested ones but the differences were not significant.

## Discussion

### Mistletoe colonization within trees

Trees infested by mistletoe showed increased recent defoliation, a drop in BAI and an enhanced responsiveness to drought

as compared with trees without mistletoe. The growth reduction in severely and moderately infested was severe since 2001, i.e., when the infestation process was probably starting to be intense. Further, the growth loss of infested trees was magnified during dry years, and it was particularly noticeable in apical wood samples. Such growth decline indicates the importance of linking growth and crown assessments with the spatio-temporal patterns of mistletoe infestation within tree crowns. The fact that moderately and severely infested trees showed similar radial growth patterns indicates that a continuum of tree infestation classes may be more appropriate for detecting mistletoe-related growth declines than using discrete classes.

Within the infested Scots pine crowns, we found that the mistletoe formed small clusters (radius  $\sim 1$  m) of similar age and that this variable and the basal diameter of mistletoe individuals increased towards the exposed crown apex. These strongly aggregated spatial patterns suggest that the first mistletoe individuals successfully establishing within the tree did it near the exposed apex and the upper crown, and then they aged and became mature producing seeds dispersed mainly by gravity within the parent host tree. Afterwards, younger mistletoes established at lower heights, probably on outer well-illuminated young branches with abundant needles, and



intra-tree colonization progressed and probably enhanced dispersion to nearby trees (Overton 1996).

- Q8 Noetzli et al. (2004) and Vallauri (1998) also detected similar clustering patterns of mistletoes in the upper third of the crown and near the main stem in the case of infested *Abies alba*
- Q9

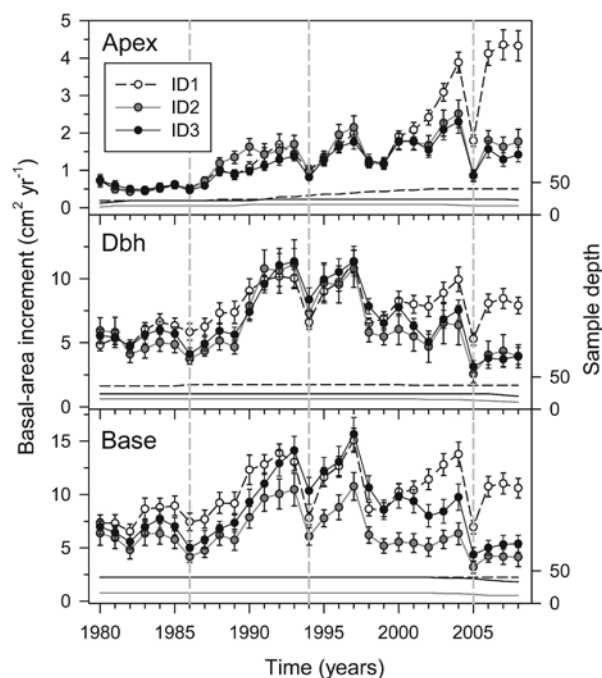


Figure 4. Growth patterns (basal area increment, means  $\pm$  SE) of Scots pine trees with different mistletoe infestation degrees (trees without mistletoe, ID1,  $n = 20$  trees; moderately infested trees, ID2,  $n = 10$  trees; severely infested trees, ID3,  $n = 10$  trees) and for wood samples taken near the apex, at 1.3 m (dbh) or near the stem base. The vertical dashed grey lines indicate selected drought years (1986, 1994 and 2005). The right axis shows the sampled depth, i.e., the number of radii measured per year (lowermost lines are of the same types and colours as basal area increment data).

Table 3. Mean ( $\pm$ SE) values of drought sensitivity (DS) and drought recovery (DR) based on radial growth data as a function of position along the stem (apex, dbh or 1.3 m, base) and the mistletoe infestation degree (ID1, no infestation,  $n = 20$  trees; ID2, moderate infestation,  $n = 10$  trees; ID3, severe infestation,  $n = 10$  trees) for one wet year before mistletoe infestation started (1983) and three selected drought years during the inferred period of mistletoe infestation (1986, 1994 and 2005). See Materials and methods for an explanation on the calculation of DS and DR.

Year	ID	Apex		dbh (1.3 m)		Base	
		DS (%)	DR (%)	DS (%)	DR (%)	DS (%)	DR (%)
1983	ID1	20.13 $\pm$ 6.16	31.36 $\pm$ 13.66	23.39 $\pm$ 7.99	29.31 $\pm$ 10.31	21.48 $\pm$ 6.78	24.13 $\pm$ 13.57
	ID2	18.36 $\pm$ 13.42	29.87 $\pm$ 18.03	22.17 $\pm$ 8.71	14.51 $\pm$ 7.05	23.08 $\pm$ 6.49	14.41 $\pm$ 7.29
	ID3	19.70 $\pm$ 14.04	28.73 $\pm$ 26.95	26.74 $\pm$ 8.55	23.61 $\pm$ 4.80	21.45 $\pm$ 7.78	23.94 $\pm$ 7.44
1986	ID1	-38.09 $\pm$ 9.05	110.23 $\pm$ 18.59	1.82 $\pm$ 3.77	27.59 $\pm$ 6.88	-7.52 $\pm$ 4.60	23.66 $\pm$ 9.82
	ID2	21.52 $\pm$ 13.47	144.50 $\pm$ 17.05	2.87 $\pm$ 9.32	37.03 $\pm$ 11.84	-11.44 $\pm$ 3.26	26.30 $\pm$ 9.48
	ID3	16.99 $\pm$ 8.16	103.40 $\pm$ 18.61	-7.88 $\pm$ 6.22	20.54 $\pm$ 7.74	-15.19 $\pm$ 4.13	17.55 $\pm$ 5.66
1994	ID1	-24.75 $\pm$ 5.06	32.32 $\pm$ 14.36	-31.48 $\pm$ 4.31	-0.10 $\pm$ 4.81	-40.20 $\pm$ 3.50	-0.70 $\pm$ 4.60
	ID2	-31.06 $\pm$ 9.86	26.59 $\pm$ 17.80	-31.35 $\pm$ 4.58	-3.32 $\pm$ 7.56	-37.09 $\pm$ 5.47	-2.75 $\pm$ 10.24
	ID3	-27.65 $\pm$ 7.81	42.48 $\pm$ 27.17	-25.79 $\pm$ 5.03	-4.50 $\pm$ 3.79	-21.17 $\pm$ 7.21	6.59 $\pm$ 7.35
2005	ID1	-40.06 $\pm$ 6.14	39.71 $\pm$ 13.30	-39.68 $\pm$ 3.54	-9.54 $\pm$ 4.39	-45.44 $\pm$ 2.54	-15.77 $\pm$ 2.85
	ID2	-59.04 $\pm$ 5.94	-26.74 $\pm$ 9.07	-59.39 $\pm$ 6.68	-37.39 $\pm$ 9.14	-50.16 $\pm$ 14.06	-34.28 $\pm$ 7.60
	ID3	-67.87 $\pm$ 6.15	-27.32 $\pm$ 10.73	-65.59 $\pm$ 3.75	-49.74 $\pm$ 5.83	-52.15 $\pm$ 3.34	-46.01 $\pm$ 3.98

and *Pinus nigra* trees, respectively. Furthermore, the increase in mistletoe age and size towards the crown apex may hold for other mistletoe–conifer interactions such as *Arceuthobium tsugense* and *Tsuga heterophylla* because mistletoe age and swelling diameter are also highly related in dwarf mistletoes (Trummer et al. 1998). In our case such patterns were established for three severely infested trees and containing on average 820 individuals per tree.

To determine if such patterns are similar in other affected conifer species further infested trees should be considered, and age structures might also be built to infer the establishment dynamics of mistletoe within individual host trees (Noetzli et al. 2004). The descriptions of within-tree spatio-temporal patterns of mistletoe infestation on tree growth at sites with contrasting climatic conditions and environmental constrains. These individual patterns should be translated to coarse scales to determine if mistletoe infestation induces changes of canopy form, stem growth and carbon uptake at the stand level (Bell and Adams 2011). This might be the case in lodgepole pine stands heavily infested by *Arceuthobium americanum* which had more foliage in the lower than in the upper canopy than lightly infested stands (Godfree et al. 2003).

Finally, although mistletoe distributions appear to be highly clumped within trees, infested trees may (Shaw et al. 2005) or may not be (Overton 1996) more clumped than the overall host population, probably as a function of bird-mediated fruit dispersal among trees and stand features. For instance, Alfaro et al. (1985) found that western hemlock trees infested by dwarf mistletoe were either included within infection loci or evenly distributed throughout the stand as a function of the pattern of the initial inoculum sources or depending on tree regeneration and the resulting stand structure.

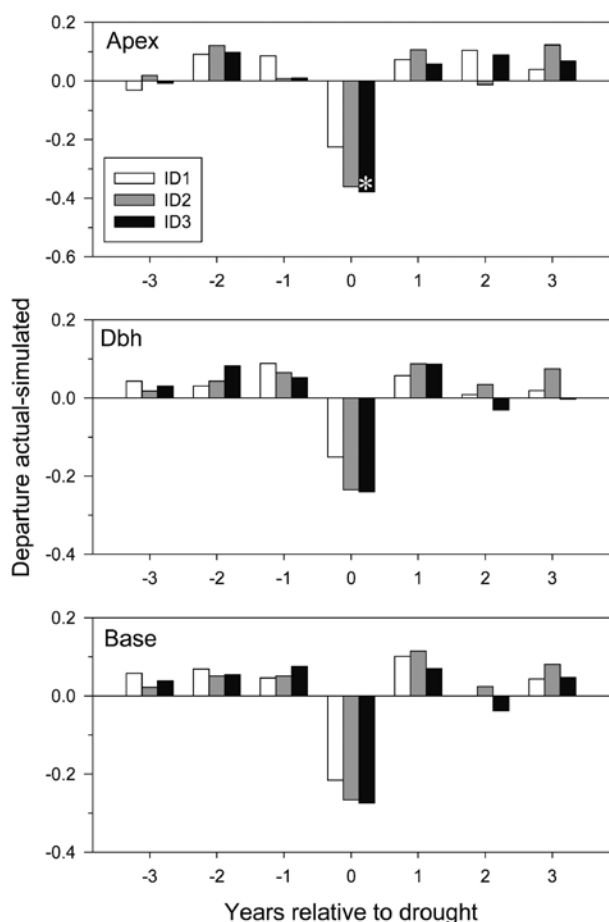


Figure 5. Standardized growth departures of Scots pine 3 years before and after the occurrence of selected droughts (1986, 1994 and 2005; see Figure 4). Growth departures were calculated considering different mistletoe infestation degrees (trees without mistletoe, ID1,  $n = 20$  trees; moderately infested trees, ID2,  $n = 10$  trees; severely infested trees, ID3,  $n = 10$  trees) and for wood samples taken near the apex, at 1.3 m (dbh) or near the stem base. Superposed epoch analysis was used to detect significant ( $P < 0.05$ , asterisk) actual growth departures from simulated data.

### Mistletoe and drought stress affect growth negatively but particularly near the tree apex

We found that BAI of severely infested trees and moderately vs. non-infested trees diverged since 2001 and this divergence was magnified by the last analysed drought in 2005. The intense increment drop in 2001 may be related with the exponential growth of mistletoe infestation within trees since most individuals reproduce when they are 7 years old (Zuber 2004). Further, the BAI drop from 1997 to 1999, when wet conditions prevailed, coincided with a major expansion of mistletoe; since then we detected a large number of 10–15 year old mistletoes (see Figure S2 available as Supplementary Data at *Tree Physiology Online*). Moderately infested trees showed lower BAI values than severely infested ones before the mid-1980s, but this trend changed after the last drought in 2005 when severely infested trees showed the lowest apical BAI values. Shaw et al. (2008) also detected that dwarf mistletoes negatively affected the growth of large western hemlock trees when trees were severely infested. Our findings that the three thirds of the crown hold abundant and usually clustered mistletoe individuals of several age classes indicate that mistletoe infestation produces detectable growth reductions once the tree is severely or moderately infested.

Moderately infested trees showed similar or lower BAI values than severely infested ones until 2005, when severely infested pines showed the lowest BAI values. This may be interpreted as a cumulative and non-linear negative effect of mistletoe infestation and successive droughts on tree growth which may react to both stressors showing threshold-type responses. In addition, moderately infested trees show the lowest BAI values before the analysed period (1980–2008), which may indicate that they were growing at lower rates than other trees because of additional constraints (e.g., micro-habitat) not related to recent mistletoe infestation. Lastly, our classification may not adequately differentiate mistletoe effects on growth between

Table 4. Descriptive growth statistics calculated for the common period 1974–2008 based on raw (first-order autocorrelation) and residual growth chronologies for the wood samples taken at three different heights (base, dbh 1.3 m and apex) in trees without mistletoe (class ID1).

Period	Base	dbh	Apex
	1914 – 2008	1929 – 2008	1968 – 2008
No. of trees (cores)	20 (40)	20 (40)	20 (40)
Mean length of tree-ring series (years)	72.3 ± 2.9	60.2 ± 2.7	27.1 ± 1.4
Mean (± SE) tree-ring width (mm)	1.58 ± 0.09	1.64 ± 0.01	1.27 ± 0.05
Mean sensitivity (MSx)	0.186	0.196	0.198
First-order autocorrelation (AC)	0.182	0.141	0.108
Mean correlation between trees ( $r_{bt}$ )	0.494	0.450	0.472
Common period <sup>1</sup>	1924 – 2008	1948 – 2008	1974 – 2008
Growth variance explained by climate (%) <sup>2</sup>	28.0	33.6	54.7

<sup>1</sup>Period with EPS higher than 0.85 (EPS, expressed population signal; see Wigley et al. 1984);

<sup>2</sup>Based on multiple linear regressions calculated between indexed ring widths and monthly climatic variables (mean temperature, total precipitation; see Figure S5 available as Supplementary Data at *Tree Physiology Online*).

Table 5. Statistics ( $F$ ,  $P$ ) of a linear mixed-effects model considering trees as random factors and comparing concentrations (means  $\pm$  SE) N, SS, starch, total NSC and C : N ratios in current-year needles or sapwood of branches from Scots pine trees without mistletoe (ID1,  $n = 10$  trees) or severely infested by mistletoe (ID3,  $n = 10$  trees). Significant ( $P < 0.05$ ) differences between infestation classes are in bold.

	Mistletoe infestation degree	N (%)	SS (%)	Starch (%)	NSC (%)	C/N
Sapwood	ID1	—	0.41 $\pm$ 0.02	0.90 $\pm$ 0.06	1.31 $\pm$ 0.07	—
	ID3	—	0.35 $\pm$ 0.02	0.84 $\pm$ 0.07	1.19 $\pm$ 0.09	—
	$F$ ( $P$ )	—	0.02 (0.90)	0.01 (0.95)	0.01 (0.93)	—
Needles	ID1	1.10 $\pm$ 0.04	5.98 $\pm$ 0.15	10.47 $\pm$ 0.42	16.45 $\pm$ 0.47	48.36 $\pm$ 0.47
	ID3	1.14 $\pm$ 0.04	5.76 $\pm$ 0.20	7.74 $\pm$ 0.31	13.50 $\pm$ 0.20	47.71 $\pm$ 0.20
	$F$ ( $P$ )	0.01 (0.98)	<b>4.51 (0.05)</b>	3.77 (0.08)	10.50 (0.09)	0.10 (0.76)

moderately and severely infested trees because mistletoe infestation and tree growth are not linearly related.

Both moderately and severely infested trees presented similar BAI patterns and showed a similar responsiveness to drought. The fact that the moderately and severely infested trees display low and high defoliation levels, respectively, suggests that mistletoe causes an increase in drought sensitivity regardless of the crown condition. Therefore, the BAI differences between non-infested and both moderately and severely infested trees would correspond to the effects of mistletoe. On the other hand, the BAI differences between moderately and severely infested trees would represent the effect of crown defoliation, which is minor as compared with the effects of mistletoe infestation on tree growth.

Final BAI was reduced by 64% near the apex in severely infested trees as compared with moderately or non-infested trees, whereas reductions were  $\sim$ 51% in the case of wood samples taken at 1.3 m or near the stem base. The finding that mistletoe infestation negatively impacted tree growth but this effect and its interaction with drought were stronger near the tree apex than at 1.3 m or near the stem base may have practical importance. For example, sampling wood cores at higher locations along the stem and near the tree apex would provide better estimates of mistletoe-induced growth decline than taking samples at the standard 1.3 m height. If such dendrochronological sampling is performed before mistletoes reach the exponential colonization dynamics within the host tree, growth data from those apical samples and defoliation estimates might also serve as forecasting tools of decline.

The negative impacts of drought on growth in severely infested trees' trends were recorded at all heights, but were only detected statistically ( $P < 0.05$ ) in apical samples where wood formation showed the highest sensitivity to water availability in spring. As in our case, Stanton (2007) also found that the growth of infested trees is more sensitive to drought stress than that of uninfested trees. Mistletoes act as additional stress factors related to drought-linked decline as has been found in Mediterranean fir forests from Greece (Tsopelas et al. 2004) and in Scots pine forests in the dry inner Swiss Alps (Dobbertin and Rigling 2006). In such xeric woodlands

mistletoe infestation seems to act combined with drought stress producing negative effects on tree growth by further decreasing both carbohydrate and water availability, making its hosts more susceptible to decline (Rigling et al. 2010).

In agreement with our data, the trees most infested by mistletoe in Pyrenean silver fir forests were dominant individuals (Oliva and Colinas 2007). This may be due to two facts: first, there is a strong positive association between mistletoe infestation and host tree height because frugivorous birds usually perch on tall trees (Aukema and Martinez del Rio 2002); second, *V. album* establishment and growth are favoured in illuminated parts of the crown (Zuber 2004), and in some cases the exposed parts have been defoliated by mistletoe thus initiating a positive mistletoe-defoliation feedback. In the study area open stands dominate and tree-to-tree competition is not high which explains why tree height and crown cover were not related ( $r = 0.01$ ,  $P = 0.79$ ). Hence, dominant trees were severely infested by mistletoe but they did not seem to correspond to a group of trees currently characterized by thin crowns and a high sensitivity to drought.

#### Mistletoe-induced growth reduction is not strongly linked to carbon and N limitations

Scots pine responded more to mistletoe infestation by decreasing wood formation than by reducing the storage of carbohydrate and N compounds in the sapwood and needles. Although we found that infestation was linked to a decrease in SS, starch and NSC concentration in the needles, such decreases did not show statistically significant differences between non-infested and severely infested trees. These data suggest that the carbohydrate status of drought-stressed Scots pine was not strongly limited by mistletoe. Perhaps, a more extreme damage due to prolonged infestation or more severe water shortage could lead to severe growth decline, abundant needle loss and carbon limitation (Galiano et al. 2011). However, mistletoe-induced decline in radial growth could also lead to a surplus in sugars being diverted towards carbohydrate storage. A reduced carbon assimilation by meristems such as the cambium may be caused by decreasing concentration of indole-3-acetic acid which favours assimilate import into sink organs (Thomas et al. 2006). This explanation concurs with the fact that BAI was

most reduced in apical wood samples taken within the crown of severely infested trees suggesting again a sink but not a source limitation. Further studies may quantify pools instead of using concentrations to evaluate differences in carbon and N storage as a function of mistletoe abundance. Taken together, our results indicate that growth decline in Scots pine after mistletoe infestation is not due to carbon (source) limitation and may be explained by a sink limitation due to reduced sink strength of cambial activity.

Mistletoes derive considerable amounts of water and carbon from infested branches and show higher transpiration rates and larger sap flow rates than the host trees (Richter and Popp 1992, Marshall et al. 1994, Ziegler et al. 2009). In fact, defoliation and growth decline have been linked to mistletoe-induced drought stress since needle development is constrained and leaf-to-sapwood ratios are modified in infested branches and trees (Sala et al. 2001, Meinzer et al. 2004, Rigling et al. 2010). High transpiration rates also allow mistletoes to accumulate more N than the host trees (Marshall et al. 1994). However, we did not find differences between sapwood from branches of non-infested and infested trees in terms of total NSC concentrations which agrees with what Logan et al. (2002) found. We observed that infested trees had lower concentrations in their needles of SS and starch which may be caused by the selective loss of old needles in infested branches (Rigling et al. 2010), by the reduction of photosynthetic rates in the host (Glatzel and Geils 2009) and by the extraction of carbohydrates from the xylem sap of the tree by mistletoe (Escher et al. 2004). The latter authors described high carbohydrate acquisition efficiency by mistletoe on an evergreen conifer host (*A. alba*) which would agree with our findings and explain the significant reduction of mobile SS concentrations in the needles of infested branches.

Our findings partially agree with those of Galiano et al. (2011) because both studies did not observe any effect of mistletoe on the carbohydrate concentrations in the sapwood. However, they concluded that mistletoe infection reduced leaf N content in needles but our data do not support that last contention despite previous studies showing that leaf N concentrations are greater in mistletoe plants than in the host tree (Reblin et al. 2006). Perhaps, in their study site trees were more stressed by drought and mistletoe than in our locality. We obtained estimates of carbohydrate concentrations similar to other healthy Scots pine forests (Hoch et al. 2003), whereas Galiano et al. (2011) measured much lower carbohydrate sapwood concentrations than us. In the study site, mistletoe infestation does not seem to drive to carbon depletion despite SS concentrations in needles decreased in branches of infested trees.

### Concluding remarks

To conclude, mistletoe infestation increased defoliation and induced growth decline in host trees. The decline in radial

growth was exacerbated by drought stress. Further, this growth decline was particularly noticeable in the stem apex. The studied forest is located near the southernmost distribution limit of Scots pine, i.e., probably close to the xeric climatic limit of the species. If drought frequency and severity increase in the study area during this century due to rising temperatures, which is the most likely climatic scenario (IPCC 2007), increasing water deficit in spring may aggravate the negative impacts of expanding mistletoe infestation causing growth decline and enhancing defoliation. Nevertheless, the role assigned to mistletoe as an opportunistic stressor (a 'contributing factor' sensu Manion 1991) negatively affecting growth of trees already weakened by drought ('inciting factor' sensu Manion 1991) should be adequately and critically evaluated. Long-term mistletoe effects on tree growth and performance in drought-prone forests may be cumulative, i.e., acting when mistletoe has invaded most of the tree crown and after successive severe droughts. Our study suggests that mistletoe may act as a 'predisposing factor' sensu Manion (1991) by predisposing trees to drought effects on tree vigour (increased defoliation and reduced radial growth), so they enter a decline process in which other pathogens, e.g., bark beetles, could ultimately cause death of the tree.

### 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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