Wood-trait analysis to understand climatic factors triggering intra-annual density-fluctuations in co-occurring Mediterranean trees

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ABSTRACT

Mediterranean trees and shrubs form intra-annual density fluctuations (IADFs) in tree rings as a sign of their plasticity in wood formation in response to intra-seasonal variations of environmental conditions. Different species show a different aptitude to form IADFs, due to their diverse ability to cope with climate stressors, since the occurrence of IADFs may affect plant hydraulics. Dendroecology and quantitative wood anatomy were used to characterise IADFs in Pinus pinea and Arbutus unedo co-occurring at a Mediterranean site in Italy. The relations between climate parameters (i.e. temperature and precipitation) and intra-annual tree-ring traits (i.e. IADF frequency and conduit size) were analysed to highlight the main triggers for IADF formation and their functional role.

Data showed that both species are characterised by a high plastic response to climate and formed a high frequency of L-IADFs (occurrence of earlywood-like conduits in latewood). The two species, although forming the same type of IADFs, showed different sensitivity to environmental factors. Pinus pinea showed a high dependence of tracheid size on temperature, while Arbutus unedo was more sensitive to precipitation in spring and autumn. Arbutus unedo promptly developed more than one IADF per year in response to rainfall events following drought periods.

The overall results were useful to compare the aptitude of the two species in forming IADFs and to highlight the factors priming their formation. This is useful to understand wood growth reactions to environmental drivers and to evaluate the adaptive capabilities in these two species, and thus to predict forest reactions after climate changes.

Keywords: Pinus pinea L., Arbutus unedo L., false rings, functional wood traits, semi-arid climate, tree rings.
INTRODUCTION

Wood research is more and more involved in trait-based ecology: there is an increasing interest in investigating the plasticity of wood anatomical traits in response to environmental constraints to evaluate the adaptive capability of trees and shrubs under climate change scenarios (Beeckman 2016). This need arises from the concern about the increasing frequency of extreme events (e.g. severe drought, heat waves, flooding) that can negatively impact wood growth, ultimately leading to tree mortality and forest decline (Sarris et al. 2007; Gea-Izquierdo et al. 2014).

The rhythm of cambial activity and dynamics of xylogenesis are genetically determined, thus species-specific: in trees growing in favourable environmental conditions, such genetic factors can be considered the main trigger for wood formation (Fisher et al. 2007; Cocozza et al. 2011; Ursache et al. 2013; Mizrachi & Myburg 2016). In the case of harsh environmental conditions, climatic factors can play a prominent role in wood formation and induce plastic adjustments in quantitative traits of conductive and non-conductive cells, which therefore record climatic trends during the growing season (Fromm 2013; De Micco et al. 2016a; Martinez del Castillo et al. 2016; Cirillo et al. 2017). In temperate environments, the tree rings formed in response to genetic and climatic factors show the typical alternation of earlywood and latewood bands due to the occurrence of one main flush of growth in spring (Fritts 1976). However, deviations in the course of xylogenesis, due to the action of abiotic and biotic factors during plant life, can determine structural “anomalies” in tree rings, called intra-annual density fluctuations (IADFs) which have been reported in many environments, including boreal and tropical, but are most frequent in species growing in Mediterranean ecosystems (De Micco et al. 2016b). Actually, in Mediterranean woods, IADFs are more the rule than the exception: their origin and functional role have been described in different species, both softwoods and hardwoods (Battipaglia et al. 2010, 2014; Campelo et al. 2013; De Micco et al. 2014, 2016a,c). The formation of IADFs is interpreted as an adaptive plant strategy to cope with the intra-seasonal alternation of favourable and stressful periods (De Micco et al. 2016b). It is likely that the ability to adjust promptly the rhythm of the cambial activity according to environmental factors in those species prone to form IADFs confers a competitive advantage under stressful climatic conditions. Tree rings with IADFs either contain bands of latewood-like cells in earlywood (E-IADFs), maintaining safety against embolism (due to narrow cells with thick walls) under limiting conditions, or earlywood-like cells in latewood (L-IADFs) allowing efficient water transport (due to large conducting cells) when environmental conditions are favourable (Sperry et al. 2006; Battipaglia et al. 2016; De Micco et al. 2016b).

IADF formation in some Mediterranean species is consequent to the bimodal xylogenesis guided by the typical double-stress due to summer drought and low winter temperatures which either induce cambium dormancy or slow down xylogenesis (Camarero et al. 2010; de Luis et al. 2011a,b; Battipaglia et al. 2014, 2016; De Micco et al. 2016b,c; Balzano et al. 2018). In the last decade, many studies have demonstrated that the relations between IADF formation and environmental fluctuations are species- and site-specific (de Luis et al. 2011b; Vieira et al. 2014; Zalloni et al. 2016, 2018a,b). In Mediterranean pines, L-IADFs are frequent and always occur in the second
half of the ring (Campelo et al. 2007, 2013). Such IADFs have been ascribed to the reoccurrence of favourable conditions of water availability in early autumn following a period of summer drought which temporarily reduces cambial activity (Abe et al. 2003; Battipaglia et al. 2010; de Luis et al. 2011a,b; Novak et al. 2013; Carvalho et al. 2015; De Micco et al. 2016c; Pacheco et al. 2016). A recent survey of IADFs in *Pinus* species at regional scale suggested the occurrence of a common climatic driver, namely abundant water availability in late summer/early autumn, for the formation of IADFs in Mediterranean pines (Zalloni et al. 2016). Also in the eudicot *Arbutus unedo* growing in Italy, *L*-IADFs were found, being positively correlated with precipitations of the period June-September. In this case, water availability induced a regrowth of the last part of the ring with the formation of wider xylem cells than in true latewood (Battipaglia et al. 2010; De Micco et al. 2016c). *Arbutus unedo*, under different microclimatic conditions formed *E*-IADFs which were localised in the middle of the ring (Battipaglia et al. 2010). *E*-IADFs were detected also in other species, such as *Erica arborea*, *Pinus pinaster* and *Pinus halepensis*, and were ascribed to reduced cell expansion caused by stomatal closure under summer drought conditions to increase wood safety against embolism (Sperry et al. 2006; De Micco et al. 2007; Vieira et al. 2010; de Luis et al. 2011a,b; Campelo et al. 2013; Battipaglia et al. 2014). In all these recent studies, the combination of dendrochronological and wood anatomical data with climate information has furnished valuable information on the response of wood growth to climate variations at the intra-seasonal scale.

The aim of this study was to assess how IADF frequency and wood anatomical traits are affected by intra-annual variations in climatic conditions in tree rings of two Mediterranean species, namely *Pinus pinea* and *Arbutus unedo*, co-occurring in Southern Italy. We analysed intra-annual variations of anatomical traits in tree-ring series, comparing rings with and without IADFs. Anatomical data were correlated with temperature and precipitation data. We aimed to: 1) assess if the two species were prone to form IADFs in the selected site, notwithstanding their different strategy in growth and water use; 2) identify which type of IADFs was formed in each of them; 3) determine whether there are common climatic drivers in the formation of IADFs in the two species, notwithstanding their different life strategies; 4) link the relations between climate parameters and intra-annual tree-ring traits with the information derived from xylogenesis. Indeed, a novelty of this study is that the ecological interpretations are based not only on the significance of statistical correlations but also on the knowledge of exact phases of xylogenesis that was analysed in the same plants in a previous investigation (Balzano et al. 2018). All data are discussed with reference to general trends in IADF formation in Mediterranean environments, in terms of their influence on plant functionality, their role in plant adaptation strategies, and consequences for vegetation dynamics.

**MATERIALS AND METHODS**

**Study area and studied species**

The study was conducted on *Pinus pinea* L. (stone pine) and *Arbutus unedo* L. (strawberry tree) plants co-occurring at a site on Mt. Vesuvius (14.42 E, 40.78 N, 200 m a.s.l.), near Naples, in Southern Italy. The site consists in a *P. pinea* plantation
forest, bordered by an area dominated by *A. unedo* shrubs and small trees. The climate is typically Mediterranean, with mean average summer (June-August) and winter (December-February) temperatures of 24.6 and 9.3 °C respectively, while mean total precipitation in autumn (September-December) and in summer is 99.2 mm and 27.6 mm for the period 1979–2015 (Fig. 1). The drought period lasts from June to August. Temperature data were derived from the E-OBS gridded datasets, with 1° spatial resolution (14.25–14.50 E, 40.75–41.00 N) (Haylock et al. 2008), while monthly precipitation data were obtained from the GPCC gridded dataset (Schneider et al. 2008), with 1° spatial resolution (14.00–15.00 E, 40.00–41.00 N) (KNMI Climate Explorer, Trouet & Van Oldenborgh 2013).

**Tree-ring analysis and IADF frequency**

In January 2016, twenty dominant and healthy trees of *Pinus pinea* (49.4 ± 6.9 cm (mean ± s.d.), diameter at breast height, 16 m height) and twenty small trees of *Arbutus unedo* (14.6 ± 4.4 cm diameter at breast height, 4–8 m height) were selected for the study. Two cores per plant were sampled at breast height of the trunk, from East and West directions, with a Pressler 5-mm increment borer. The cores were dried and polished; tree rings and IADFs were visually identified (Stokes & Smiley 1968). Then tree-ring width (TRW) was measured using a LINTAB system, with a resolution of 0.01 mm and synchronised using GLK statistical tests (Gleichläufigkeit, t-test). Individual series were visually cross-dated and then the program COFECHA (Holmes 1983) was run to find potential errors, before developing tree-ring chronologies. The chronologies were detrended, using the smoothing spline approach with a 50% frequency response cutoff to remove non-climatic signals (Fritts 1976) and then individual chronologies were averaged using the Dendrochronology Program Library in R (dplR; Bunn 2010).
IADFs were visually detected in 20 trees in each species. The occurrence and position of IADFs within the annual rings (i.e., within earlywood or latewood) were recorded and IADFs were classified according to De Micco et al. (2016b). IADFs of a single year were considered only when present in both cores (Kuo & McGinnes 1973). Microphotographs of tree rings of *P. pinea* without (Fig. 2a) and with (Fig. 2b, c) IADFs. Figure 2. Light microscopy views of cross sections of tree rings of a–c: *Pinus pinea* and d–f: *Arbutus unedo*. – a: Tree rings without IADFs, b: with $L^{-}$-IADF and c: $L^{+}$-IADF/transition tracheids. – d: Tree rings without IADFs, e: with $L^{-}$-IADFs and f: with double $L^{-}$-IADFs. – Yellow arrows indicate the beginning of earlywood-like cells in the latewood, documenting different types of IADFs. – Scale bars = 500 µm.
are shown. In the rings without IADFs, tracheid size varied along the ring following the usual trend, narrowing lumen and thickening cell walls moving from earlywood to latewood. IADFs were classified as: 1) \( L^- \)-IADFs, consisting in earlywood-like cells in the latewood (Fig. 2b), and 2) \( L^+ \)-IADFs or transition wood, consisting in earlywood-like cells between the latewood and the earlywood of the successive tree ring (Fig. 2c); \( E(eearlywood) \)-type IADFs were not present in either species.

In \textit{A. unedo}, tree rings showed the typical characteristics of diffuse or semi-diffuse porosity (InsideWood 2004-onwards; Wheeler 2011) depending on the year; however, latewood was clearly distinguishable in the last quarter of the ring width because of the occurrence of distinctly narrower vessels that made this wood classifiable more as semi-diffuse porous in most analysed rings (Fig. 2d). In this hardwood species, IADFs were classified only as \( L^- \)-IADFs (Fig. 2e); sometimes tree rings formed more than one \( L^- \)-IADF, appearing as two or more successive bands of earlywood-like cells in latewood (Fig. 2f).

The relative frequency of IADFs per year was calculated as the number of trees that presented IADFs in a given year, divided by the total number of sampled trees in that year. Stabilised IADF frequencies were calculated according to Osborn \textit{et al.} (1997) as \( F_{\text{stab}} = F \times n^{0.5} \) where \( F \) is the relative frequency of IADFs per year and \( n \) is the number of trees sampled that year.

\textbf{Microscopy and quantitative wood anatomy}

Cross sections (15 \( \mu \)m thick) were cut with a sliding microtome from the five cores considered more representative in terms of cross-dating and GLK per species. The sections were stained with a solution of safranin (0.04\%) and astra blue (0.15 \%) in water (van der Werf \textit{et al.} 2007), and mounted in immersion oil for microscopy (De Micco & Aronne 2007).

Sections were analysed under a transmitted light microscope (BX60, Olympus) and microphotographs of each tree ring were taken with a digital camera (CAMEDIA C4040, Olympus) and analysed with the software AnalySIS 3.2 (Olympus). In \textit{Pinus pinea}, tree rings of the years from 1979 to 2015 were analysed for a total of 130 tree rings without IADFs and 50 with IADFs. In \textit{Arbutus unedo}, tree rings from the year 2001 to 2015 were analysed for a total of 26 tree rings without IADFs and 49 with IADFs. From each microphotograph, corresponding with a tree ring, transects extending throughout the whole tree ring width were selected which included at least three radial lines of tracheids in \textit{P. pinea} and four lines of vessels in radial multiples in \textit{A. unedo}. Tracheid and vessel lumens were automatically detected in these transects, starting from the beginning of earlywood towards the ending of latewood. Lumen area of all encountered conduits (tracheids in \textit{P. pinea} and vessels in \textit{A. unedo}) was measured following the \textit{in continuum} method reported in De Micco \textit{et al.} (2012). In \textit{A. unedo}, we did not consider the size of fibres given that a previous study showed that the intra-annual trend of lumen size variation in fibres was exactly the same as in vessels; moreover, other wood functional traits were not as sensitive as conduit size and were disregarded (De Micco \textit{et al.} 2016a). In brief, during the measurement, the progressive number of each element was recorded moving within the transect from
earlywood to latewood and used to standardise the position of each conduit in terms of distance from the beginning of the ring, the whole ring width being considered equal to 100%. For each of the considered parameters, a dispersion graph was drawn, where each conduit was characterised by two coordinates: the measured lumen parameter (Y) and the relative distance from the beginning of the ring (X), expressed as percentage of the total ring width. The patterns of tracheid/vessel size variability along ring width were visually compared in rings with and without IADFs. In the standardised data series, interpolation equations (fourth-order polynomial curve) with confidence intervals (95%) were calculated using the option non-linear curve fitting in R (R Development Core Team 2018). The intercept values in the best-fitted model equation for *P. pinea* rings with and without IADFs were 1.908e+01 and 1.460e+01 respectively, while $R^2$ values were 0.269 ($P < 0.01$) and 0.324 ($P < 0.01$) respectively. The intercept values in the best-fitted model equation for *A. unedo* rings with and without IADFs were 2.740e+01 and 2.193e+01 respectively, while $R^2$ values were 0.198 ($P < 0.01$) and 0.151 ($P < 0.01$) respectively.

**Relations with climate**

For the calculation of correlations between conduit size and climate variables, each tree ring without IADFs was partitioned in four regions (each region corresponding to the 25% of total ring width). In tree rings with IADFs, L- and $L^+$-IADFs have been considered additional growth layers of wood according to Campelo *et al.* (2007, 2010) and Zalloni *et al.* (2018a). Therefore, in tree rings with IADFs, we made the 80% ring width (corresponding to the end of true latewood) coinciding with 100% ring width of tree rings without IADFs. In detail, tree-ring width in the presence of IADFs was divided into five regions (each region corresponding to the 20% of total ring width). The first four regions were considered coinciding with those of rings without IADFs and relative data were pooled; the fifth region was considered the wood band containing the IADF (both $L^-$ and $L^+$-types), formed later in the season if compared with rings without IADFs), as demonstrated in a previous xylogenesis study (Balzano *et al.* 2018). Mean values of tracheid/vessel lumen area were computed per each region of the ring and the correlations with maximum temperature and total precipitation of months from September of the previous year to December of the current year were calculated using the Pearson coefficient ($p < 0.05$). We selected the aforementioned months for analysis according to the cambial activity calendar of both species reported in Balzano *et al.* (2018). To avoid losing information, we considered the conditions also of the previous three months to the current year. The relationships between stabilised IADF frequency (considering both $L^-$ and $L^+$-IADFs separately) and climate data were calculated as well using the same method. In the analysis, we included conduit chronologies of five trees per species and the chronology of stabilised IADF frequencies considering the period 1979–2015 for *Pinus pinea* and 2000–2015 for *Arbutus unedo*.

All correlations were calculated using the treeclim R package (Zang & Biondi 2015).
RESULTS

Tree-ring dating and IADF occurrence

*Pinus pinea* chronologies have a mean length of 96 years (Fig. 3a), with expressed population signal (EPS) of 0.94 and GLK >60, showing high correlation between the mean chronology and the radial growth variation of individual trees within the population. The r bar value, which is a measure of the strength of the signal, was 0.35. The mean TRW was 2.87 and 2.19 mm in rings with and without IADFs respectively. *Arbutus unedo* plants were younger than *P. pinea* trees (Fig. 3b), with average chronologies of 20 years, EPS of 0.96, GLK >60, and r bar of 0.53, which indicated a good synchronisation between individual trees. The mean TRW was 3.42 and 3.16 mm in rings with and without IADFs respectively.

We found that the IADFs were present in the 28.01% and 68.27% of total rings in *P. pinea* and *A. unedo* respectively. In particular, in *P. pinea* the mean stabilized IADF frequency was 1.20 and 0.13 for L- and L+-IADFs respectively; in *A. unedo* it was 3.17 and 0.16 for L- and double-IADFs, respectively.

Lumen area chronologies of conductive elements

In the first half of tree rings of *Pinus pinea*, earlywood cells were characterised by larger lumina in rings with IADFs when compared with those without IADFs (Fig. 4a). The two curves coincided at 50–70% of the ring width. Then, tracheid size continued to decrease in rings without IADFs, while it increased again in rings with IADFs which showed larger earlywood-like tracheids in the latewood region (at 80–100% of ring width). The variability between rings was lower in rings without IADFs but the correlation coefficient was similar in the two types of rings (R = 0.516, p < 0.01, for rings with IADFs; R = 0.569, p < 0.01, for rings without IADFs).
In *Arbutus unedo*, at the beginning of the ring (until about 10% of the ring width), earlywood vessels were characterised by larger lumina in rings with IADFs when compared with those without IADFs (Fig. 4b). Vessel size progressively decreased in rings without IADFs from earlywood to latewood. Conversely, in tree rings showing IADFs, vessels size showed a first non-significant decrease at a distance of about 20% from the beginning of the ring and a second, more severe, significant decrease in the region around 50–70% of the ring width, compared to rings without IADFs. Vessel lumen size increased again in rings with IADFs reaching maximum values in the LW region (at about 90% of ring width). The variability between rings and the correlation coefficient were similar in the two types of rings (r = 0.445, p < 0.01, for rings with IADFs; R = 0.388, p < 0.01, for rings without IADFs).

**Climate relations**

Climatic analysis at the monthly scale revealed a different influence of temperature and precipitation on the lumen area of conductive elements and on IADF occurrence in both species. In *Pinus pinea*, tracheid lumen area was more sensitive to the maximum temperature (Fig. 5a, p < 0.05) than precipitation (Fig. 5b). Lumen area of tracheids at the beginning of earlywood (Region 1) was positively correlated with April maximum temperature (p < 0.05); moreover the whole earlywood (Regions 1, 2) was positively affected by the maximum temperature of the previous autumn months and April (Fig. 5a, p < 0.05) while Region 3 (transition wood) was negatively correlated with the precipitation of August and December (Fig. 5b, p < 0.05). Tracheids of latewood (Region 4) were negatively influenced by the maximum temperature of May (Fig. 5a, p < 0.05). Lumen area of the IADF region (Region 5) was positively influenced by maximum temperature of April and December (Fig. 5a, p < 0.05) and by precipitation in June (Fig. 5b, p < 0.05).
In *Arbutus unedo*, a positive correlation was found between lumen area of earlywood vessels (Region 1) and temperature in April (Fig. 5c, p < 0.05). Vessels between earlywood and transition wood (Region 3) were positively influenced by April temperature (Fig. 5c, p < 0.05) and precipitation in February and October (Fig. 5d, p < 0.05). Vessel lumen in the latewood (Region 4) was negatively correlated with February maximum temperature (Fig. 5c, p < 0.05) and positively correlated with previous year December and current year January, February and October precipitation (Fig. 5d, p < 0.05). Lumen size in the IADF region (Region 5) was negatively correlated with January maximum temperature (Fig. 5c, p < 0.05) and March precipitation (Fig. 5d, p < 0.05), and positively correlated with April and July precipitation (Fig. 5d, p < 0.05).

Correlations between climatic data and stabilised IADF frequencies in *P. pinea* showed a negative relation between the occurrence of *L*-IADFs and precipitation of December of the previous year (Fig. 6a, p < 0.05). A positive relation was found with the maximum temperature of August and October (Fig. 6b, p < 0.05). The occurrence of *L*-IADFs was instead correlated negatively with precipitation of the previous October (Fig. 6c, p < 0.05) and positively with the temperature of December (Fig. 6d, p < 0.05).

Regarding *A. unedo*, *L*-IADF occurrence was positively correlated with precipitation in September of the current year (Fig. 6e, P < 0.05) and negatively correlated with maximum temperature of the previous September and current August (Fig. 6f, p < 0.05). Double *L*-IADF occurrence was positively correlated to precipitation of
Figure 6. Climate–IADFs associations computed by comparing IADF frequency and climate parameters (precipitation, in black, and maximum temperature, in grey, in the graphs on the left and right columns respectively) in a, b, c, d: *Pinus pinea* and e, f, g, h: *Arbutus unedo*. Relations between precipitation and the frequency of a: L-IADF and c: $L^+$-IADF in *P. pinea*; maximum temperature and the frequency of b: L-IADF and d: $L^+$-IADF in *P. pinea*; precipitation and the frequency of e: L-IADF and g: double-IADFs in *A. unedo*; maximum temperature and the frequency of f: L-IADF and h: double-IADFs in *A. unedo*. Correlations were calculated from September of the previous year to December of the current year of tree-ring formation. Significant correlations (p < 0.05) are indicated by asterisks.
January, March, June and November (Fig. 6g, p < 0.05) and maximum temperature of April (Fig. 6h, p < 0.05), while it was negatively correlated with precipitation of August (Fig. 6g, p < 0.05) and maximum temperature of June (Fig. 6h, p < 0.05).

DISCUSSION

The analysis of tree rings of *Pinus pinea* and *Arbutus unedo* growing at the same site in Southern Italy showed that the two species, although having completely different wood models, namely of softwood and hardwood, and having different life strategies and age, react to the constraints of the Mediterranean climate through the formation of the same type of IADFs. Indeed, both species form earlywood-like conducting cells in latewood in agreement with previous studies, thus confirming the steadiness of the formation of L-IADFs as a qualitative response to abundant precipitation after periods of drought (De Micco et al. 2016b; Zalloni et al. 2016). However, further analysis of IADF frequency, intra-annual anatomical traits and relations with climate also demonstrated that the two species have different degrees of xylem plasticity. *Arbutus unedo* seems to be more sensitive to variations of climatic fluctuations in so far as it shows a higher frequency of IADFs, together with the occurrence of more than one IADF per year. Moreover, in *A. unedo*, the increase in vessel size found in the last part of late-wood was preceded by a significant decrease in the previous sector of the ring which is not evident in *P. pinea*. The higher sensitivity of *A. unedo* compared to *P. pinea* can be due not only to a genetic predisposition, but also to the younger age of the plants. Indeed, younger plants generally show wider tree ring width which in turn is correlated with higher IADF frequency (Bogino & Bravo 2009; Vieira et al. 2009; Novak et al. 2013; Campelo et al. 2015). The young age of the *A. unedo* plants can be also related to a shallow root system, likely being more sensitive to fluctuating water availability in upper soil layers (Cherubini et al. 2003).

The L-IADFs found in the two species suggest the occurrence of a bimodal cambial activity pattern which is considered an adaptive strategy of Mediterranean plants to cope with low winter temperature and summer drought (Camarero et al. 2010). The bimodal cambial activity in *P. pinea* and *A. unedo* was confirmed by the analysis of xylogenesis in the year 2015 which showed the occurrence of two main peaks of cambial activity in spring and autumn, alternating with two moments of cambial dormancy or slowed xylogenesis in winter and summer (Balzano et al. 2018).

Considering the stabilised frequency of IADFs in *P. pinea*, the main drivers for the formation of L-IADFs appeared to be the high temperatures in August and October, together with low precipitation in the previous winter. The presence of high water availability in the soil would promote wood growth and the formation of wider rings which are more prone to form IADFs in agreement with the mechanism proposed for L-IADF formation in many *Pinus* species growing in Mediterranean areas (Zalloni et al. 2016). The positive relation with temperature in August and October, together with low precipitation in the previous winter, suggests that their occurrence is related to summer drought (induced by the combination of high temperature and low precipitation) and to warm temperature in October which, in the absence of water limitations thanks
to autumn rains, would promote again the development of earlywood-like cells. This hypothesis is in agreement with xylogenesis data which demonstrate the formation of IADFs in the middle of October (Balzano et al. 2018). In a recent study, Zalloni et al. (2016) reported that the increase in temperature throughout the year plays an important positive role for L-IADF formation in P. pinea from a network across the Mediterranean Basin. Moreover, a study on P. pinea in Portugal showed that stabilised frequency of L-IADF is related to high temperature in March and May and precipitation in October and November (Campelo et al. 2007). The important role of temperature on xylem differentiation has also been recently highlighted by several studies on conifers growing in the Northern Hemisphere (Castagneri et al. 2018; Björklund et al. 2018). This variability of results confirms that, although triggered by the same mechanism, IADF formation in the Mediterranean region is site-dependent and presents a high year-to-year variability, strongly dependent on climate (Vieira et al. 2014).

The positive relation found between IADF frequency and the temperature of December suggests that warm conditions in late autumn/winter play a role also for the formation of $L^+$-IADFs. Such conditions would allow the prolongation of wood formation, avoiding the usual period of winter cambium dormancy (de Luis et al. 2011a; Campelo et al. 2013). Vieira et al. (2009) also reported positive correlations between December temperature and $L^+$-IADF frequency in P. pinaster suggesting that cells can continue to differentiate until the end of the year or even all year round. Moreover, latewood tracheids in P. halepensis were found to be still differentiating in December, demonstrating that warm winters could extend the period of xylogenesis (de Luis et al. 2011b; Novak et al. 2016). Xylogenesis data reported in Balzano et al. (2018) support that the formation of $L^+$-IADFs, consisting in the so-called transition tracheids, occurred in winter months, when temperature was not below the threshold inducing cambial dormancy. The positive correlation between December temperature and tracheid lumen size supports the idea of the winter temperature as a trigger for the formation of earlywood-like cells after the normal latewood production. Temperature, more than precipitation, guided tracheid size in the earlywood in P. pinea. Warm conditions in April, at the beginning of true earlywood formation (Balzano et al. 2018), possibly determine the differentiation of tracheids with wide lumina. This suggests that, at the beginning of the active growing season, air temperature has the main influence on cambium activity and cell enlargement, while precipitation is not a crucial factor, probably because the soil moisture supply is not limiting after the winter/spring period (Antonova & Stasova 1997).

Lumen area of latewood tracheids in P. pinea is negatively affected by the increase in the temperature of May. As reported in Balzano et al. (2018), the production of latewood cells in P. pinea started at the beginning of June. This suggests that latewood is formed as a response to the increasing temperature of May: high temperature would be a signal to induce the formation of narrower tracheids to improve safety of water transport (Sperry et al. 2006). The significant correlation between wet conditions in June and the occurrence of larger lumen tracheids formed in autumn in the regions of the L-IADFs suggest that summer rain events can promote the formation of earlywood cells in a second peak of cambial activity in autumn, probably due to the positive role
played in soil refilling. Finally, the negative relation between the amount of precipitation in some months (i.e. August and December) and tracheid size in latewood may be explained also considering the relative rates between cell enlargement and cell wall deposition which ultimately regulate conduit size and morphology (Cartenì et al. 2018). Under conditions of high water availability but either too high (in August) or too low (in December) temperature, resource allocation would be more shifted towards cell wall deposition rather than to cell proliferation and enlargement, thus leading to narrower conduits characterised by thicker cell walls.

As regards Arbutus unedo, stabilised frequency of L-IADFs seems to be positively related to precipitation in September. This is in agreement with findings in the same species growing at a site in Central Italy, where positive correlations found between the stabilised frequency of IADFs and the June-September precipitation suggested such rain events as drivers needed to allow the species to resume xylem production in autumn after summer dormancy (Battipaglia et al. 2010). Moreover, the findings that the occurrence of double L-IADFs was positively correlated with precipitation in March, June, and November, while it was negatively correlated with precipitation in August, suggest the role played by fluctuating water availability during the year to activate/accelerate and deactivate/slow down cambial production more than once a year. The occurrence of more than one flush of xylem growth during the calendar year is in agreement with previous findings in A. unedo and with xylogenesis data (Cherubini et al. 2003; De Micco et al. 2016c; Balzano et al. 2018).

As in P. pinea, also in A. unedo the temperature in April was positively correlated with conduit size of both earlywood and latewood, suggesting the role of this factor not only on the onset of xylogenesis (Balzano et al. 2018), but also on the process of cell enlargement. Vessel size in latewood was also positively controlled by precipitation in February and October which would assure enough water reserves in the soil, especially before the summer drought period and in the period of IADF formation (Balzano et al. 2018). Vessel size in the region of the IADF showed the more numerous significant correlations with precipitation, either positive or negative, suggesting a valuable role of water availability in modulating cell production, enlargement and differentiation promptly at the intra-annual scale. A previous study in southern France already highlighted the sensitiveness of A. unedo to precipitation in the months April-July showing a positive tree-growth response (Nijland et al. 2011) and indicating a high dependence of the trees on rainfall during these months. The high sensitivity of A. unedo to precipitation, suggested by the correlations with climatic parameters, indicates that the species is very adapted to the frequent intra-seasonal variations of water availability and shows high plasticity in xylem formation. This is in agreement with previous studies where this species was demonstrated to form different types of IADFs in study sites characterised by different soil water availability (Battipaglia et al. 2010).

In conclusion, the overall analysis showed that both species are characterised by highly plastic response to climate, although with different degrees and timing. P. pinea trees were demonstrated to be able, as other Mediterranean pines, to sustain low growth rates during the summer dry period and winter (Camarero et al. 2010; De Micco et al.
The low dependence of tracheid size on precipitation suggests that the trees of this species can reach deeper soil-water stocks and are less influenced by short rain events when compared to *A. unedo*. On the other hand, *A. unedo* appeared more sensitive to precipitation in spring and autumn and was able to respond promptly to subsequent rainfall events during these periods, also with the formation of more than one IADF per year. These results demonstrate that, although forming the same type of IADFs, species co-occurring at the same site can show different sensitivity to environmental factors. Therefore, main triggers for IADF formation can vary according to the species.

The different ability of different species to cope with environmental constraints by modulating intra-annual variation of xylem anatomy determines their efficiency and safety against embolism (Sperry *et al.* 2006) and therefore affects their growth. Consequently, the aptitude to form IADFs, being a sign of different capability of different species to perform plastic adjustment of xylogenesis, is crucial for plant adaptation and survival, and ultimately may influence vegetation dynamics in a climate change scenario.

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