Report: "**Intra-Annual-Density-Fluctuations in Mediterranean pines: how do they change with species, geographical distribution and climate?**"

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1. Introduction & Purpose of the STSM

Intra-Annual-Density-Fluctuations (IADFs) are abnormal changes in wood density that occur within the growth rings of the woody species (Fritts, 1976). Several studies attest the correlation between IADFs formation and climatic conditions (Battipaglia et al., 2010; Campelo et al., 2007; de Luis et al., 2007, 2011; De Micco et al., 2012; Wimmer et al., 2000). Thus, the identification of the fluctuations provides information on the relationship between environmental factors and eco-physiological processes of trees. These structures can be useful to understand the impact that fluctuations in precipitation and temperature can have on tree growth during the growing season. Since tree response to climate can vary among sites and/or species (de Luis et al., 2013; Nabais et al., 2014; Novak et al., 2013; Rigling et al., 2001, 2002), the impact of climate changes can vary considerably depending on the ability that different species have to adapt to the different environments in which they live. Moreover, IADFs can occur in the earlywood and/or in the latewood, with different anatomical features which lead us looking for different related environmental signals.

The aim of this STSM was precisely to understand how climate and tree species affect IADFs formation, in terms of frequency and position along the tree ring, comparing

ring-width and IADFs chronologies of two of the main Mediterranean pine species (*Pinus pinea* and *Pinus pinaster*) growing along a latitudinal transect in Italy, Spain and Portugal to different climate conditions. The analysis of the results of this STSM are expected to enable us i) to understand what climatic conditions trigger IADFs formation and frequency, ii) the differences between species and site and iii) the contribute of the increasing drought stress on IADFs.

2. Description of the work carried out during the STSM

Five sites from Spain, three sites from Italy and one from Portugal were analized in order to reach the purpose of the work. Two Mediterranean pine species were considered: *P. pinea* from two sites from Spain and from the three Italian ones, and *P. pinaster* from Spain (3 sites) and Portugal (1 site). A map of the studied sites and species is represented in Figure 1. Informations about the sites are showed in Table 1.

Figure 1. The studied sites. **Se.E** = Serra de Estrela; **Des** = Parque Natural Despeñaperros; **Ru.A** = Rubieros Altos; **C.Pus** = Parque Natural de Cazorla – Puerta de Segura; **Si.H** = Parque Natural Sierra de Huetor; **Mo.M** = Montes de Malaga; **Du.F** = Duna Feniglia; **Fre** = Fregene; **Cas** = Castelporziano.
PIPI = *Pinus pinaster*; **PIPN** = *Pinus pinea*.

Table 1. Informations about the studied sites.

Site	Country	Species	Last year of growth	N Trees	N Samples	Altitud (m.a.s.l.)	Latitude (°)	Longitude (°)
P.N. Despeñaperros	Spain	*Pinus pinaster*	2012	10	20	600	38.374745	-3.513931
Montes de Malaga	Spain	*Pinus pinea*	2012	9	17	135	36.763873	-4.418169
P. N. Cazorla – Puerta de Segura	Spain	*Pinus pinaster*	2012	10	20	1030	38.362212	-2.720307
Rubieros Altos	Spain	*Pinus pinea*	2012	12	23	800	39.480271	-2.046686
P.N. Sierra de Huetor	Spain	*Pinus pinaster*	2012	11	21	1120	37.272933	-3.543662
Serra de Estrela	Portugal	*Pinus pinaster*	2010	26	56	1000-1100	40.38	-7.55
Castelporziano	Italy	*Pinus pinea*	2003	21	21	0-20	41.7403	12.4016
Duna Feniglia	Italy	*Pinus pinea*	2003	14	14	0-20	42.4425	11.2223
Fregene	Italy	*Pinus pinea*	2012	22	22	0-20	41.8558	12.2006

The spanish samples were prepared and analysed at the University of Zaragoza, while the ones from Portugal and Italy were processed at the University of Coimbra.

All the cores from Spain (two cores per tree) were first sanded and then cutted with a core-microtome (Gärtner H. & Nievergelt D., 2010) to produce a flat surface on which tree-ring boundaries and density fluctuations were clearly visible. Then, all samples were scanned and different photos taken for each core. These photos were used to cross-date the samples of each site with the software CooRecorder (http://www.cybis.se), which allows to identify tree-ring boundaries and helps the detection of missing rings and fluctuations. The software was useful to have an easier and accurate comparison between cores visually compared on the screen. Tree-ring width, earlywood and latewood widths of cross-dated samples were then measured with an accuracy of 0.01 mm, using the linear table Lintab (Frank Rinn S.A., Heidelberg, Germany) and the program TSAP-Win (Rinn, 2003). Then, presence/absence of density fluctuations within each ring of all the cores was determined on dated cores using a stereomicroscope, attributing to each tree ring 1 for the presence and 0 for the absence.

The samples from Serra de Estrela (two cores per tree), in Portugal, were already sanded and prepared for the study. While the cores from the three Italian sites (one core per tree) had to be sanded.

Tree-ring width, earlywood and latewood widths of all of them, except the site of Castelporziano (a chronology was already available), were cross-dated with the help of the program TSAP-Win (Rinn, 2003) and measured with an accuracy of 0.01 mm, using the linear table Lintab (Frank Rinn S.A., Heidelberg, Germany).

Then, presence of density fluctuations within each ring of all the cores was determined at a stereomicroscope, and a visual identification of the type of the fluctuation was done, following the classification proposed by Campelo et al. 2007 for *P. pinea* (Figure 2).

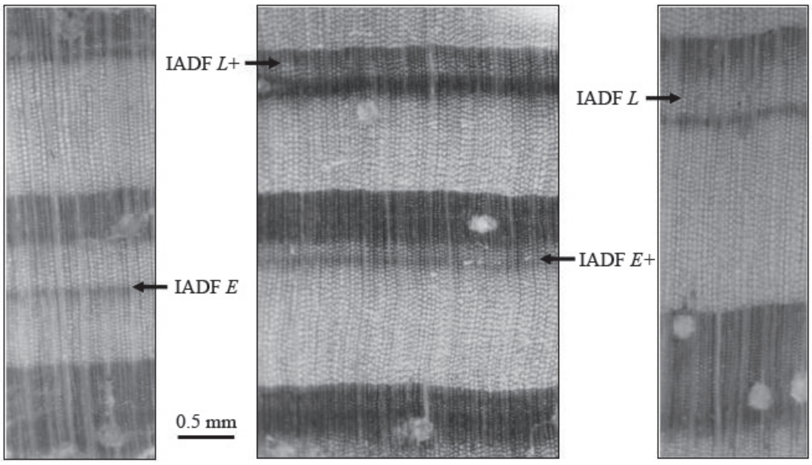

Figure 2. Different types of IADFs in *Pinus pinea.* **Type E**: latewoodlike cells within earlywood; **Type E+**: transition cells between earlywood and latewood; **Type L+**: earlywoodlike cells between latewood and earlywood of the next tree ring; **Type L**: earlywoodlike cells within latewood. (Campelo et al., 2007)

Finally, to remove age-related growth trends and competition a detrending was applied to the series fitting a smoothing cubic spline function of 60 years to the longer chronologies (Castelporziano, Fregene, P.N. Cazorla – Puerta de Segura), 40 years to the chronologies of medium lenght (Duna Feniglia, Rubieros Altos, Serra de Estrela, P.N. Sierra de Huetor), and of 30 to the shorter ones (P.N. Despeñaperros, Montes de Malaga), using the packages dplR (Bunn, 2008) and detrendeR (Campelo et al., 2012) of the R freeware program (http://www.cran.r-project.org). A biweight robust estimate of the mean was applied to reduce the influence of isolated outlier values, and an autoregressive model was fitted to the standardized indices to remove the previous year effect. Descriptive statistics of the obtained residual chronologies were produced.

Stabilized frequency (Osborn et al, 1997) of each different type of fluctuation was calculated for the sites with this classification available; only stabilized frequency of presence was calculated for the sites from Spain.

The IADFs stabilized frequencies were correlated with monthly climate data of mean temperature and total precipitation obtained from the nearest 0.5° x 0.5° grid point at http://climexp.knmi.nl/ for each site, using Pearson's correlation coefficients. Correlations were calculated for the time span 1951-2000, from August of the previous year to December of the current year, in order to search for any influences of temperature and precipitation oscillations on the current year of growth, relative to the previous growing season.

3. Description of the main results obtained

Hereby a selection of preliminary results obtained during my STSM is presented, with some introductive notes.

1) IADF frequencies were calculated using cores (when only one core is available per tree) or trees (when two cores per tree were available; in this case a fluctuation was only considered when both cores from the same tree showed an identical fluctuation in the same ring).

2) The correlation between climate and frequencies of each IADF type was possible only for half of the dataset. For the remaining part, correlations were calculated only between climate and frequencies of presence data, without considering IADF types.

3) The different applied procedures were mainly due to different methods of processing samples used in the different laboratories. This was an important point of discussion during the Workshop 5 of the COST meeting in Estoril (24-25 October 2014) which I attended during my STSM, with the future aim of making this kind of procedure uniform in order to be able to correctly compare IADFs data on a large geographical area.

Only tree-ring width, and not earlywood and latewood widths, was taken into account in this preliminary analysis. The expressend population signal (EPS) for all the residual series was higher than the threshold value of 0.85. The common period of 1951-2000 in which all the individual cross-dated series of all the sites showed an EPS value > 0.85 was selected for the analysis, in order to obtain a strong climate signal in the chronologies.

Main results:

IADFS frequency: Considering the data of presence/absence of all the types of fluctuations together and taking into account all the sites, IADFs were more frequent in tree rings of the cores of *P. pinea* from the Italian coastal site of Duna Feniglia (50.4%), followed by *P. pinaster* from Serra de Estrela (Portugal) with the value of 40% of frequency. While the lowest percentage of IADFs presence was registered for *P. pinea* from the Spanish inland site of Rubieros Altos (1%), followed by *P. pinaster* from another inland site of Cazorla – Puerta de Segura (Spain) with 2.7%.

The overall frequencies show that *P. pinea* and *P. pinaster* from the Spanish sites formed significantly less fluctuations compared to *P. pinea* from the Italian coastal sites and to *P. pinaster* of the Portuguese site. The site of Despeñaperros was the only one from Spain showing an higher frequency of 25.1% of fluctuations. (Table 2)

Table 2. Outputs of the analysis of the overall frequency of IADFs presence. (BC) means that the analysis was performed per tree (considering IADF presence only when present in both cores of a tree).

Site	N of rings analysed	N of rings with IADFs	Frequency
P.N. Despeñaperros	490	123	25.1% (BC)
Montes de Malaga	1125	77	6.8% (BC)
P. N. Cazorla – Puerta de Segura	1250	34	2.7% (BC)
Rubieros Altos	996	10	1% (BC)
P.N. Sierra de Huetor	781	32	4.1% (BC)
Serra de Estrela	2021	809	40% (BC)
Castelporziano	2174	623	28.7%
Duna Feniglia	958	483	50.4%
Fregene	2441	623	25.5%

IADFs classification: Regarding the sites for which the classification of the type of fluctuation was available (the Portuguese and Italian ones), most of the IADFs found in both species and in all the sites were located in the latewood, both L and L+ type. There were no fluctuations of type E while a low frequency of E+ type, often in association with an L or an L+ type fluctuation in the same growth ring. The maximum frequency value reached by the presence of the E+ type was only of 0.9% (Castelporziano site). Thus, no correlation between this IADF type and climate was calculated, except for the Portuguese site of Serra de Estrela, where E+ type frequency was higher than 8%.

Climate correlations: Preliminary results coming out from the correlations between IADFs frequencies and monthly mean temperature and total precipitation data, show the positive effect that precipitation of the period from October to November of the current year have on the formation of the IADFs of type L in the rings of the coastal sites from Italy and of September on the fluctuations of type L+ in the inland site of Portugal, sometimes associated with high temperatures and a low amount of precipitation during August and September. While correlations showed an effect of the amount of precipitation of September on the formations of the IADFs for most of the sites from Spain. Again, where the classification of the types were available, the distinction between different climate signals was possible. While for the other sites, a single mixed signal was showed. Positive correlations with mean temperatures of March and May or with precipitations of April associated with a negative correlation with temperatures of July in the case of the E+ type formation in the Portuguese site were also found.

Further statistical analyses will be performed to improve the climate signal and to enable a better distinction between the different responses of sites and species. Moreover, additional analysis methods will be applied to remove the effect of size and age on the formation of IADFs, as suggested by recent papers suggesting that IADF formation and climate-growth relationship is size-mediated (i.e. Campelo et al., 2013 and de Luis et al., 2009) and age-mediated (Novak et al., 2013 and Vieira et al., 2009). Results will be published in a scientific paper in an international scholarly journal, which will also serve as an output for the COST Action FP1106 STReESS.

4. Description about how the results contribute to the Action aims

This "STReESS STSM" has contributed to the creation and organization of a quite large IADFs dataset, that was an important issue raised during the Workshop 5 of the last COST meeting in Estoril (24-25 October 2014). Results improved the Intra-Annual-Fluctuations network which needs data from different latitudes in order to shed light on the relationships between climate and fluctuations. This STMS strengthened the collaboration between the Action members of the Italian, Spanish and Portuguese groups, which is one of the main aim of the inter-laboratory exchange visits.

5. Confirmation by the host institutions of the successful execution of the STSM

The confirmation letter by the host institution of successful execution of the STSM is attached in a file.

6. Authorization to post the report at the Action website

We authorize to post this report at the Action website.

7. References

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