Report: "Ecophysiological responses of *Pinus canariensis* to Canary Island volcanic eruption through dendrochronological and stable isotope analyses"

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1. Introduction/Purpose

Disturbances in tree growth due to volcanic eruptions have been widely studied (Battipaglia *et al* 2007; Brifa *et al* 1998; LaMarche and Hirshboeck 1984). During eruptions, tephra and aerosols may be ejected in high enough quantities to cause locally to worldwide climatic variations lasting a few years, depending on the eruptive intensity. Such environmental perturbations shape the vegetation structure after the eruption.

The Canary Islands, have suffered 14 eruptions since 1490, two of them occurred in the last century in La Palma island. The main forest-forming species in the island is the Canary Island pine (*Pinus canariensis*) a distinctive taxon in the genus due to its resprouting capability. Among other characteristics, resprouting enables this species to deal with strong environmental disturbances. Furthermore, a great quantity of this Canary Island pines managed to survive in the surroundings of the Hoyo Negro's crater after its eruption in 1949. This pine trees suffered not only by the climatic variations due to the eruption, but were also directly damaged by ashes, lapilly and bombs. They were thus critically damaged and remaining trees were reduced to an injured crown-less bole.

Four trees were selected among individuals with scars and damaged crown structures in the affected area. In 2010 and 2013 these trees were fallen and slices were sampled aiming to identify the physiological mechanisms that allowed their survival.

The study of certain anatomical features and isotopic signatures of wood yields information on tree physiological and ecological responses to different sources of stress. These techniques have been previously used to understand eruption effects on vegetation (Battipaglia *et al* 2007; Tognetti *et al* 2012). Although the trees in those studies had been affected in a distinct way, those studies showed the applicability of dendrochronology and carbon and oxygen stable isotopes analysis to determine the tree conditions in pre- and post-disturbance stages.

Thus, the aim of this Short-Term Scientific Mission (STMS) was to allow the candidate to learn and perform the techniques of dendrochronology and stable isotope analyses. The use of such techniques will help understanding tree resilience to extreme environmental perturbations, specifically on tree responses to volcanic eruption damages.

2. Description of the work

The analyses were performed in a total of 20 *Pinus canariensis* cross-section samples that were transported to Second University of Naples in Caserta. Those samples belonged to the four trees previously sampled (5 slices per tree). The slices were already sanded and prepared for the study.

Firstly, a dendrochronological analysis was performed. Three radius symmetrically distributed were measured per slice, in order to minimize the effect of the reaction wood present in most of the samples. Ring width measurements were made with a resolution of 0.01 mm, using LINTAB measurement equipment (Frank Rinn, Heidelberg, Germany) fitted with a Leica MS5 stereoscope and analysed with TSAP software package. The ring width series were plotted and visually synchronized for identification of errors during the measurements and for potential missing or double rings (Fritts, 1976; Schweingruber, 1996). As the aim of this work was to study the effects of the eruption in these trees, we didn't create a master chronology but a chronology per each tree. Cross dating of the tree-ring series in each tree was verified using the cross dating package implemented in the TSAP software, which assesses the quality of cross dating and measurement accuracy of tree-ring series using the segmented time-series correlation technique (Holmes, 1983). When cross-dating errors were indicated for the collected data, we went back to the cross-sections and determined the possible source of error. Individual series that did not cross date were first compared with the two other radiuses of the same tree and then with the mean ring-width of the tree. When they were not coincident and corrections could not be made, the problematic tree-ring series was removed. Then, to determine and remove long-term trend without altering or modifying the existing data, the first differences method (1 year minus the previous one $DY_t=Y_t-Y_{t-1}$) was applied to all measurements.

To run the stable isotope analyses we pooled the rings every five years from 1940 to 1969 in each tree. This allowed us to have two groups of rings before the eruption in June 1949 as control values and 4 groups of rings after it. Not all 4 trees had the same number of effective groups (groups of years with tissue growth allowing isotopic signature measures), as there were different numbers of missing rings depending on the tree. We selected one cross section per tree to be sampled. The selected slices where those which presented less missing rings after the eruption. We only sampled the slices in one radius as the stable isotope ratio remains constant around the circumference of each ring (De Micco *et al*, 2013).

The samples were ground with a centrifugal mill (ZM 1000, Retsch, Germany) using a mesh size of 0.5 mm to ensure homogeneity. Cellulose was extracted with a double-step digestion (Boettger *et al*, 2007; Battipaglia *et al*., 2008) with the Teflon pocket method. 50 mg of powdered sample of each group was measured and stored in Teflon pockets sealed with a heat sealer. The first step consisted in the treatment of wood with a solution of 5% NaOH for 2 h at 60 ºC, repeated twice, in order to remove lipids, resins, oil, tannins and hemicelluloses. In the second step, samples were washed with a 7% $NaClO_2$ solution for 36 h at 60 1C. Because the solution is only reacting for 10 h, this step was done in 5 days in 7-8 hours cycles. Finally, samples were washed three to four times with boiling distilled water and dried overnight at 50 ºC. Sub-samples of the obtained cellulose of 0.06 mg and 0.4 mg were measured and stored in tin and silver capsules for analyzing C and O isotopes respectively.

Figure 1: Sample preparation process. a) Dendrochronology study; b) sampling 5 ring groups from the slices; c) centrifugal mill; d) and e) double step digestion to obtain cellulose; f) cellulose of one group of pooled rings; g) prepared tin capsules with a sample of cellulose for δ13C analysis

The C isotope values were measured by combustion and the oxygen isotopes by pyrolysis of the cellulose samples in an elemental analyzer (Carlo Erba 1110, Milano, Italy) interfaced via a Conflo II Interface (Thermo Finnigan, Bremen, Germany) to a dual inlet/continuous flow isotope ratio mass spectrometer (Delta S, Thermo Finnigan) operating in the continuous flow mode. The isotopic analyses were performed at the CIRCE laboratory (**C** enter for **I** sotopic **R** esearch on the **C** ultural and **E** nvironmental heritage, Caserta, Italy).

The isotope signature is expressed in the δ notation for carbon and oxygen, where $\delta^{13}C$ or $\delta^{18}O=(R_{sample}/R_{standard}-1)$ (‰), relative to the international standard, which is VPDB (Vienna Pee Dee Belemnite) for carbon and VSMOW (Vienna Standard Mean Ocean Water) for oxygen. R is the ratio of heavy to light isotopes. The standard deviation for the repeated analysis of an internal standard (commercial cellulose) was lower than 0.1 ‰ for carbon and 0.2 ‰ for oxygen. The calibration vs. VPDB was carried out by measurement of IAEA-C3 that has an $\delta^{13}C$ certified value of -24,91 ± 0,49 (‰) and vs VSMOW by measurement of USGS32 that has an $\delta^{18}O$ certificated value of -25,70 ± 0,4 (‰)

Data provided by Francey et al. (1999) and McCarroll and Loader (2004) were used to remove the decline in the δ13 C of atmospheric CO_2 due to fossil fuel emissions from the carbon isotope data series. The corrected series were then employed in all the statistical analyses.

3. Description of the main results obtained

I hereby present the preliminary results obtained during the STSM.

Dendrochronology

The dendrochronological study of *Pinus canariensis* usually presents some difficulties (Jonsson *et al* 2002). This species is very plastic and easily generates false or missing rings. Two slices were discarded (one of tree T1 and another of tree T4) because it was not possible to crossdate the series with the whole data. The T1 discarded cross-section, was a basal slice that might have been influenced by root growth. The T4 discarded slice, was part of a second bole, as the tree was divided in two trunks from its base.

The analysis of the ring-widths, showed a great variability in tree-ring growth between trees. The analysis also indicated that trees T1, T2 and T4 were mature trees during the eruption while T2 was only 20 years old in 1949.

A constant pattern was observed in all trees. In 1949 after the eruption, there was a period in which no growth was observed. The four trees also showed a decrease in mean growth after the eruption.

Stable isotopes

As previously stated, there was a different number of effective groups, depending on the tree (Table 1)

Table 1: Distribution among the trees of the effective groups (groups of years with tissue growth allowing isotopic signature measures)

		Year	T1	T2	T3	T4
After eruption	G1	1964-1969	X	X	X	X
	G2	1960-1964	X	X		X
	G3	1955-1959		X		X
	G4	1950-1954				X
Before eruption	G8	1945-1949	X	X	X	X
	G9	1940-1944	X	X	X	X

There were not significant differences in $\delta^{13}C$ values in pre and post-eruptive groups. On the other hand, $\delta^{18}O$ values showed a significant decrease for at least 10 years after the eruption, turning back into pre-eruptive levels after that period. There were not significant correlations between both isotope signals, as a result of the lack of variation in $\delta^{13}C$.

These preliminary results show *Pinus canariensis* as a tree that deals with the stress in a "passive way". Trees stop their radial growth immediately after the damage. And years later, when the trees are recovered, they are able to restart their activity, without showing any sign of stress in $\delta^{13}C$. $\delta^{18}O$ instead changes after the eruption showing a difference in the post-eruption tree activity that may be related to changes in the crown.

Further statistical analysis of the results and deepest interpretation of their meaning will be performed after the STSM. The results will be presented at appropriate local and international conferences and we expect to produce a manuscript to be submitted to a rated-peer reviewed journal.

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

In this STSM two principal objectives had been accomplished:

- Through applying dendrochronology and stable isotope analyses to the sampled trees, we have contributed to enhance the understanding of tree responses to extreme stress situations, in particular stress triggered by eruption damages. The study species seems to deal with stress by having a "latency period" without growth, restarting its activity when pre-disturbance conditions are re-established. The observed response to stress is unusual among European trees, and thus it increases the interest of our study in understanding the mechanisms involved.

- Moreover, during my STSM I've learned a new technique (stable isotope analysis), that will open new approaches in the studies that are taking place in my research group in Spain. So, beyond the results expected after this STMS, future collaborations between both groups will be possible thanks to this opportunity.

5. Confirmation by the host institution 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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