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1           **Evaluation of electrical signals in pine trees in a**  
2           **Mediterranean forest ecosystem**

3  
4   Rodolfo Zapata, Jose-Vicente Oliver-Villanueva, Lenin-Guillermo Lemus-Zúñiga, Jorge E.

5   Luzuriaga, Miguel A. Mateo Pla and Javier F. Urchueguía

6   Universitat Politècnica de València, ITACA - Institute of Information and Communication

7   Technologies, Research Group ICT against Climate Change, València ,Spain

8  
9   \* Corresponding author:

10   Rodolfo Zapata

11   Universitat Politècnica de València

12   ITACA - Institute of Information and Communication Technologies

13   Research Group ICT against Climate Change ([ictvscc.webs.upv.es/en/](http://ictvscc.webs.upv.es/en/))

14   Camí de Vera s/n

15   46022 València (Spain)

16   email: rozaza@upv.es

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18

1 **ABSTRACT**

2

3 **Evaluation of electrical signals in pine trees in a Mediterranean forest ecosystem**

4

5 Electric potential differences in living plants are explained by theories based on sap  
6 flow. In order to acquire more advanced knowledge about the spatial distribution of  
7 this electric potential measures in trees, this research aims to analyse electrical signals  
8 in a population of Aleppo pines (*Pinus halepensis* Mill.) in a representative  
9 Mediterranean forest ecosystem.

10

11 The specific research objective is to assess some of the most significant factors that  
12 influence the distribution pattern of those electric signals: tree age, measurement type  
13 and electrode placement.

14

15 The research has been conducted in representative forest stands, obtaining  
16 measurements of different representative trees. After a statistical evaluation of the  
17 obtained results, the main conclusions of our research are:

18

- 19 A. Tree maturity influences directly on electric potential.  
20 B. Maximum electrical signals can be measured in young pines showing values  
21 of 0.6 V and 0.6  $\mu$ A for voltage and current, respectively.  
22 C. The distribution patterns of both voltage and short-circuit current depending  
23 on electrode placement are uniform.

24

25

26 **Keywords:** natural electric signs; plant electrical potential; natural electrical power;  
27 Mediterranean pines; tree age influence; electrode placement influence; adaptive  
28 forest management.

29

30

# 1 1. INTRODUCTION

2 Plants have electrical activity; this fact was first revealed by Burdon-Sanderson <sup>[1]</sup> and  
3 Darwin <sup>[2]</sup> by observing insectivorous plants. Bose <sup>[3]</sup> and then Pickard <sup>[4]</sup> evidenced the  
4 existence of action potentials in some plants regardless of the presence of rapid leaf  
5 movements. Regarding trees, Wright and Fisher <sup>[5]</sup> detected the existence of a  
6 difference in continuous electrical potential between the electrodes inserted in the  
7 tree's phloem and the surrounding soil.

8 There are both, physical and biological hypothesis to explain the origin of the electrical  
9 signals measured in plants that have been scientifically documented.

10 On the one hand, the biological hypothesis establishes that different parts of the  
11 plants develop in their evolution an internal system <sup>[11]</sup>. Following this, the  
12 transmission of electrical signals at the cellular level activates physiological responses  
13 to external environmental stimuli <sup>[11]</sup>. In this context, different studies have reported  
14 the effect of different stimuli, such as light conditions <sup>[12,13]</sup>, temperature variations <sup>[14-</sup>  
15 <sup>18]</sup>, and mechanical wounds <sup>[19, 11]</sup>.

16 According to some authors <sup>[7,11,20-22]</sup> the variation of the electrical signals depends on  
17 the intensity of the stimulation that could be associated with changes in water tension  
18 or ion concentrations, creating a transient electrochemical imbalance. These electrical  
19 signals would be transmitted through living cells of plants. Understanding mechanisms  
20 of electrical responses, changes in physiological processes and predicting responses to  
21 stress factors requires mathematical modelling of electrical activity in plant organisms,  
22 a review of such models can be found in Sukhova<sup>[23]</sup>.

23 On the other hand, the physical hypothesis is based on the fact that trees continuously  
24 suck water, nutrients and charged particles into the xylem by transpiration <sup>[6,7]</sup>, In this  
25 process a pH imbalance is created between the tree and the soil, which causes an  
26 electric current that circulates between different parts of the trees <sup>[6]</sup>. Several  
27 experiments to support this hypothesis are available in different studies, e.g., the  
28 phloem <sup>[7]</sup>, the xylem and the leaves <sup>[6]</sup>, as well as other elements of the living tree and  
29 the adjacent soil <sup>[8]</sup>.

30 Another physical theory is based on the fact that the electric current is the result of  
31 electrokinetic phenomenon, which is caused by the movement of liquids in a porous  
32 medium <sup>[9,10]</sup>. In order to model the physical models behind this potential, it is  
33 necessary to perform the experiments under the controlled conditions of a laboratory.  
34 Additionally, several works have been done with agricultural species under laboratory  
35 conditions <sup>[11,31-33]</sup>. We also have to take into account that some of these studies are  
36 limited only to analysing just the voltage data <sup>[10, 27]</sup>.

1 All these requirements make it difficult the researches about trees in forests, which  
2 could explain the reduced number of publications found. For example, the studies in  
3 [6,8,24,25] observed electrical signals in individual trees, which do not allow analysing the  
4 variability between individuals in an ecosystem. Furthermore, the few investigations  
5 that we have been able to document have been carried out mainly in Continental and  
6 central European climatic areas, e.g. *Aesculus hippocastanum* [26] and *Populus nigra* [10]  
7 both in France, *Eucalyptus globulus* in Portugal [26], *Salix alba* in Austria [28], *Quercus*  
8 *spp.* and *Fagus sylvatica* [29,30].

9 Consequently, rigorous analysis of electrical signals has not been found related to,  
10 neither coniferous species nor Mediterranean ecosystems. For these reasons, the  
11 present study aims to fill this lack of knowledge. Focusing on the characterization and  
12 analysis of the electric signals under natural conditions (outside laboratories) exploring  
13 the behaviour of the two components of electrical signals (voltage and short-circuit  
14 current (ISC)) in a representative population of Aleppo pines (*Pinus halepensis* Mill.) in  
15 the Mediterranean area.

16 A suitable application of these electrical signals measures is the idea of using plants as  
17 biosensors. This idea has been studied by other authors in research laboratories under  
18 controlled conditions [31-34]. In order to use trees in the wild forest as biosensors we  
19 have to face the problem of the lack of information on electrical signals in natural  
20 uncontrolled conditions. Thus, we consider it necessary to create a good research base  
21 on the behaviour of those electrical signals of trees, both statically and dynamically.

22 In the present work, we have evaluated the most significant static factors that  
23 influence on the distribution of voltage and ISC electrical signals, specifically: i) the  
24 influence of tree age, ii) the electrode placement (height and orientation of the  
25 assignment) and iii) the measurement settings (bipolar or referenced to ground  
26 setups).

27

28

## 2. MATERIAL AND METHODS

Next, we describe the principal material and methods used in this study, which was carried out outside laboratory in representative pine trees belonging to a Mediterranean forest.

### 2.1 Sample stands

Conifer species are very representative of the Mediterranean area, and one of the most important of them is the Aleppo pine (*Pinus halepensis* Mill.)<sup>[35-39]</sup>. We surveyed a series of potential forests for this study using the layers for the Geographical Information System (GIS) of the Spanish National Forest Inventory<sup>[40]</sup>. We look for pole and pure mature stands of *Pinus halepensis* in Eastern Spain. Because of the different physiological behaviour of the trees according to the age<sup>[7,41]</sup>, we include the tree age as a critical variable in the research design and in the sample stands selection. Thus, to capture a representative tree population in these forests, we considered the following forest characteristics:

- A. An arboreal population of the same species (pure stands),
- B. Enough specimens for each age group,
- C. A not affected area (at least in the last years) by substantial environmental disturbances (forest fires, pests or snow damages).

Crossing all GIS layers with these considerations a representative sample forest was found in the *Sierra Calderona Natural Park* located in the region of Valencia, Spain. The chosen area, of approximately 20 hectares, contains pure stands of Aleppo pine trees. The trees clearly show that they belong to two different age classes, located very close to each other. These trees maintain the same edaphological conditions as well as insolation regime, orientation, and water availability. The two age classes are:

- A. Young trees: about 26-year-old naturally regenerated stands originated after a forest fire in 1994<sup>[42]</sup>.
- B. Mature trees: even-aged pure stands, which were afforested around 50 years ago and survived forest fire of 1994.

The selected sample stand has an approximate size of 0.25 hectares and is located at latitude 39° 45' 28.80" N and longitude 0° 30' 36.36" W. Figure 1 shows the nature of the study area.



Figure 1: Study area location: (A) Panoramic view of the sample stand, (B) tree distancing in the sample stand.

## 2.2 Selection of representative trees

In the selected sample stand, a total of 121 trees were inventoried. The main dendrometric parameters, i.e. diameter at breast height (DBH at 1.3 m) <sup>[43]</sup> and total tree height ( $h_t$ ) have been measured. The results of this inventory are summarised in Table 1.

	Breast-height diameter DBH [cm]	Total tree height $h_t$ [m]
<b>Mean</b>	<b>12.10</b>	<b>5.16</b>
Median	11.10	5.13
Maximum	25.10	9.69
Minimum	6.4	2.77
Standard deviation	3.95	1.30
Variance	15.59	1.68
Number of samples	121	

Table 1: Dendrometric parameters of the tree population: breast-height diameter (DBH) and total tree height ( $h_t$ ).

Following the recommendations of Fernández-Puratich <sup>[44]</sup>, Oliver-Villanueva <sup>[45]</sup> and Hapla <sup>[46]</sup>, we disregarded from the study those trees that: i) are located at the edge of the plot, ii) have grown with excessively braided shafts or iii) show affections of fungi or insects.

Furthermore, following the recommendations of Ríos-Rojas <sup>[33]</sup>, for a first phase of the study, we selected randomly different standard samples: two mature trees, two young

1 trees and a standing dead tree. This phase was focussed on voltage and current  
2 measurements as a function of tree age.

3 In the second phase of the study, we conducted a statistical study to determine the  
4 population size following the methodology described by Hapla and Saborowski <sup>[47]</sup>.  
5 These authors made several measurements at different heights of the trees  
6 demonstrating that if the height and diameter of the trees do not differ more than a  
7 confidence interval the increasing number of samples does not improve the results  
8 significantly. For this phase, our tree population was reduced to 15 representative  
9 trees, to reach 95% confidence interval, considering the selection criteria used in  
10 different studies related to wood characteristics and physical properties of several tree  
11 species <sup>[45, 46, 48-52]</sup>. The selected trees accomplish the following characteristics:

- 13 1. Height and diameter do not differ more than a standard deviation of the  
14 mean values.
- 15 2. Belong to KRAFT sociological class I (dominant trees) or class II (co-dominant  
16 trees).
- 17 3. Without significant or appreciable damage or disease present on the trunk
- 18 4. Homogeneously distributed throughout the entire plot surface, to achieve a  
19 greater diversity of results.
- 20 5. Belong to the same age group and with enough level of electrical activity  
21 (measured in phase 1 of the study)

## 22 **2.3 Measurement equipment**

23  
24 The electrical parameters were measured with a UT71D UNI-T multimeter with an  
25 input impedance of 2.5 GΩ, and accuracy of 0.1% ± 2 millivolts (mV), as it is used in  
26 other studies <sup>[53, 54]</sup>.

27 We used extracellular stainless-steel electrodes as in previous works <sup>[10]</sup>. To eliminate  
28 as far as possible the measurement overpotentials induced by the galvanic effect in  
29 bipolar measurements, we made sure that all our electrodes were manufactured with  
30 the same material.

31 We chose screws as the electrodes because they can easily be inserted into and  
32 extracted from trees, causing a small injury. Besides, because of their thread, screws  
33 have a larger contact surface area than smooth electrodes.

34  
35 For the measurements, as a ground connection to the circuit, we used a titanium  
36 nitride-coated platinum-iridium alloy electrode (1999/5/EC) <sup>[55]</sup>. Non-polarisation  
37 electrodes were used as earth to minimise false results induced by the charges freely  
38 crossing over between the electrode-electrolyte interface following the experiences of  
39 Prutchi <sup>[56]</sup>. Each electrode was connected with the measuring equipment through an  
40 electrical connector and a 0.5 mm copper conductor cable insulated with a flexible  
41 plastic coating (CE 0123). Figure 2 shows the materials and equipment used.

42



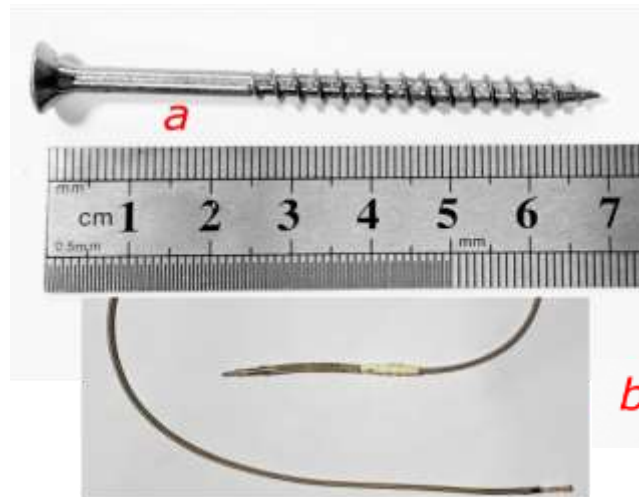


Figure 2: Equipment used for the measurement of the electrical signals (a) a 70 mm screw as an electrode, (b) an earth electrode.

#### 2.4 Electrode placement and measurement

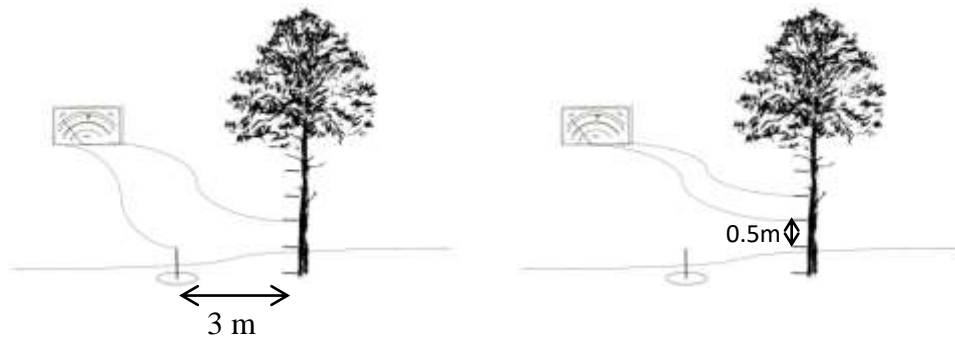
To record the voltage and short-circuit current (ISC) of the sample tree, electrodes were inserted directly into the trunk in contact with the phloematic tissue. This was ensured by inserting the electrodes using a torque wrench that allowed us to detect the change in hardness. According to other authors, phloem<sup>[11]</sup> play an active role in the electrical response of the trees. Oyarce<sup>[11]</sup> demonstrated that the living cells that compose these areas offer lower resistance to the current flow in comparison with the rest of the tissues of the plant.

We placed the electrodes in a circular and longitudinal distribution to determine the distribution of the potential difference along the trunk of each tree. In the circular ring cuff configuration, the electrodes were placed at 1.5 m above the ground at the four cardinal compass points (north, east, south and west). Using the compass to select the orientation of the faces of the trees where the electrodes were inserted, as is shown in Figure 3 (left and middle). In the longitudinal configuration, the electrodes were longitudinally inserted into the trees every 50 cm, following a longitudinal line at the trunk's north face. Figure 3 (right) shows this setup. The low-polarisation electrode was buried 15 cm into the ground, a minimum of 3 m away from the tree to use as ground or reference electrode.



Figure 3 Electrode placement using a compass (left), identification of trees and radially location of the electrodes (centre), electrodes separated longitudinally (right).

1 The electrode placement used was the same in bipolar mode or referenced to the  
 2 ground, changing only the electrodes connection. So, measurements referenced to the  
 3 ground were carried out between each electrode concerning to the ground. On the  
 4 other hand, bipolar measurements were made between two nearby electrodes, with a  
 5 fixed separation of 0.5 m between them. The first level was from the electrode located  
 6 at zero meters to 0.5 m, the next level was from 0.5 m to 1 m, continuing thus, up to  
 7 three meters in height on the tree. Figure 4 shows a diagram of this configuration.



8  
9

10 Figure 4: Electrode placement setup modes (left) measurements referenced to the ground, (right)  
 11 bipolar setup.

12 The setup used for the electrodes to measure the potential difference was the same  
 13 for the total population of trees. Between 18<sup>th</sup> and 19<sup>th</sup> November 2017, continuous  
 14 measurements were carried out during 24h, sampling data every hour. In this  
 15 measurement session, we did not intend to observe a difference in behaviour, it was  
 16 only to obtain voltage values between the two age groups.

17 After obtaining the results of the preliminary phase of the study, the trees that formed  
 18 the population for the second phase were selected, based on the observed results. The  
 19 voltage and intensity began to be measured in the 15 selected trees that made up this  
 20 representative population, analysing the distribution of the electrical potential in the  
 21 trees.

## 2.5 Experimental conditions

Data collection was performed through measurements by inserting electrodes into the trees. With the intention that our collected data were not affected by external variables, the following two premises were followed.

First, given that we caused a small injury in the trunks by inserting the electrodes, the trees responded by forming border areas and compartmentalisation barriers to prevent infection and to resist the spread of wood decay <sup>[57]</sup>. Generally, within 24 days' post of the wound creation, an impervious boundary appears approximately 1 mm inside the wound surface <sup>[57, 58]</sup>. In conifer trees, the impermeability arises between 28 and 30 days after injury in these trees <sup>[57]</sup>. Following this, we carried out our study after leaving enough time for the generation of the boundary zone (necrofilactic periderm) of the tree bark.

Second, we have to address the fact that environmental conditions affect the behaviour of the plant and generate changes in the electrical potential <sup>[25, 33, 60, 61]</sup>. To avoid that uncontrollable external factors that may influence our observations, we made the measurements of the fieldwork in days with similar air humidity and temperature, mainly in bright days without clouds. All measurements were made in the hour of zenith in the work area (between 1:30 pm and 2:00 pm CET). The decision to carry out the measurements at this time was based on the highest solar incidence, the lowest cloud presence and stable conditions.

## 2.6 Statistical Analyses

Each collected data of voltages and ISC were analysed. We carried out normal distribution, homoscedasticity of variance and ANOVA tests. In our case, to check if the data follows a normal distribution t-Student parametric test was applied. This allows evaluating the comparison between the two tree ages as well as between bipolar and referenced to the ground electrode configuration data (for two samples).

The concept of data homoscedasticity implies constancy in the variance of the errors, and it is used to guarantee a variable prediction by calculating the average value of its set <sup>[60]</sup>.

An analysis of the variance (ANOVA) was performed with north, east, south, and west (N, E, S, W) orientation data (for three or more samples). Besides, the Kruskal-Wallis test and Mann-Whitney U test were applied to check the heterogeneity of data for two and three samples, respectively.

1 **3. RESULTS**

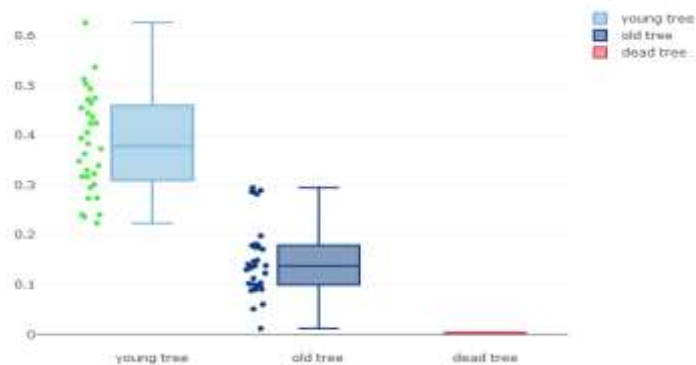
2 **3.1 Influence of tree age**

3  
 4 Table 2 shows the obtained results for young and mature trees. Furthermore, this  
 5 table also shows a significant influence of the tree age in the referenced to the ground  
 6 measurements. Hence, there is a significant difference between the age groups ( $p$ -  
 7 value  $<0.0001$ ) with an average voltage of 0.929 V in young trees and 0.238 V in  
 8 mature trees. On the other hand, the bipolar measurements show an average voltage  
 9 of 0.010 V and 0.014 V for young and mature trees, respectively. In bipolar  
 10 measurements, the age difference does not show significant differences between  
 11 groups ( $p$ -value = 0.45).

Measurement	Group	Max	Min	Mean	Std. Dev.
Referenced to the ground measurements	Young	-0.887	-1.016	-0.929	0.024
	Mature	-0.222	-0.261	-0.238	0.013
Bipolar	Young	0.033	0	0.010	0.008
	Mature	0.148	0	0.014	0.027
Mean of referenced to the ground measurements 24h	Young	-0.223	-0.626	-0.383	0.100
	Mature	-0.012	-0.295	-0.150	0.073

13  
 14 Table 2: Observations summary using different configurations over mature and young tree groups, volts  
 15 as unit of measurement.

16  
 17 Figure 5 shows the main results obtained in 24 hours of referenced to the ground  
 18 measurements. The average voltage in young trees is 0.383V with a maximum value of  
 19 0.626V. Mature trees show significantly lower values versus young trees ( $p$ -value  $<$   
 20 0.0001) with an average voltage of 0.150 V and a maximum value of 0.295 V. The  
 21 control dead tree shows values of 0 V, as expected.



22  
 23  
 24  
 25  
 26 Figure 5: Boxplots presenting the data dispersion for young, old, and dead tree 24h measurements. The  
 27 median voltages measured significantly differed according to the tree age, young trees showing much  
 28 higher values than older individuals.

1 **3.2 Influence of height**

2

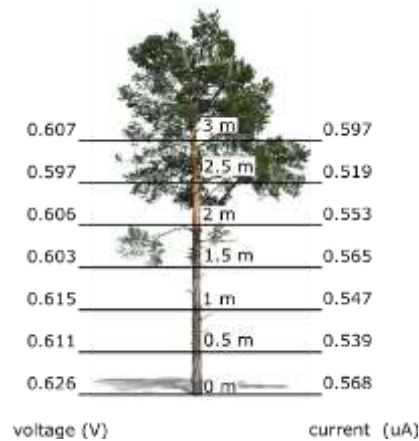
3 For the analysis of the longitudinal variation of the electric signals, only the young  
 4 trees have been selected, since they show significantly higher values in the voltage  
 5 measurements as the older trees.

6 **3.2.1 Referenced to the ground measurements**

7

8 Figure 6 and Table 3 show values very constant at every height level. With a maximum  
 9 voltage value of 0.626 V measured at ground level and a minimum value of 0.597 V at  
 10 2.5 m. The ISC show values even closer to each other than the voltage, with a  
 11 maximum ISC value of 0.568  $\mu$ A at 0 m and a minimum value of 0.519  $\mu$ A measured at  
 12 2.5 m.

13 Analysing the data obtained, we found significant differences neither in voltage (*p*-  
 14 value = 0.86) nor in ISC (*p*-value = 0.91) among the different height levels. Therefore,  
 15 the measurement height shows no influence on the voltage or ISC referenced to the  
 16 ground measurements.



17  
18

19 Figure 6: Monopolar referenced to the ground measurements for voltage and current considering the  
 20 height at the tree

21

Height (m)	Voltage (V)		ISC ( $\mu$ A)	
	Mean	Variance	Mean	Variance
0.0	-0.626	0.011	-0.568	0.027
0.5	-0.611	0.008	-0.539	0.038
1.0	-0.615	0.005	-0.547	0.023
1.5	-0.603	0.006	-0.565	0.023
2.0	-0.606	0.003	-0.553	0.027
2.5	-0.597	0.005	-0.519	0.022
3.0	-0.607	0.004	-0.597	0.021

22  
23  
24

Table 3: Statistical mean and variance of referenced to the ground measurements for voltage and current considering the height at the tree

### 3.2.2 Bipolar measurements

Both the voltage and the ISC show very close values between each height level for bipolar measurements. The highest values for voltage mean have been obtained between 1 m to 1.5 m above the ground (0.036 V) and the minimum values at 2.5-3.0 m (0.025 V). On the other hand, the maximum values for ISC were measured between 0.0 m to 0.5 m (0.471  $\mu$ A) and the minimum values at 2.0-2.5 m (0.218  $\mu$ A).

Nevertheless, the slight differences observed are not significant among levels of height, neither for voltage ( $p$ -value = 0.517) nor for ISC ( $p$ -value = 0.248).

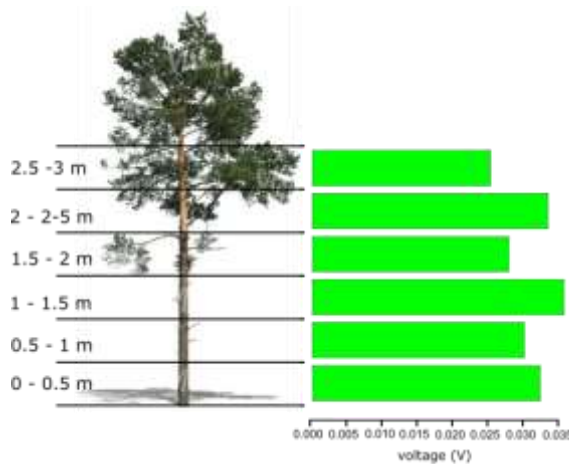


Figure 7: Bipolar voltage measurements considering the height at the tree.

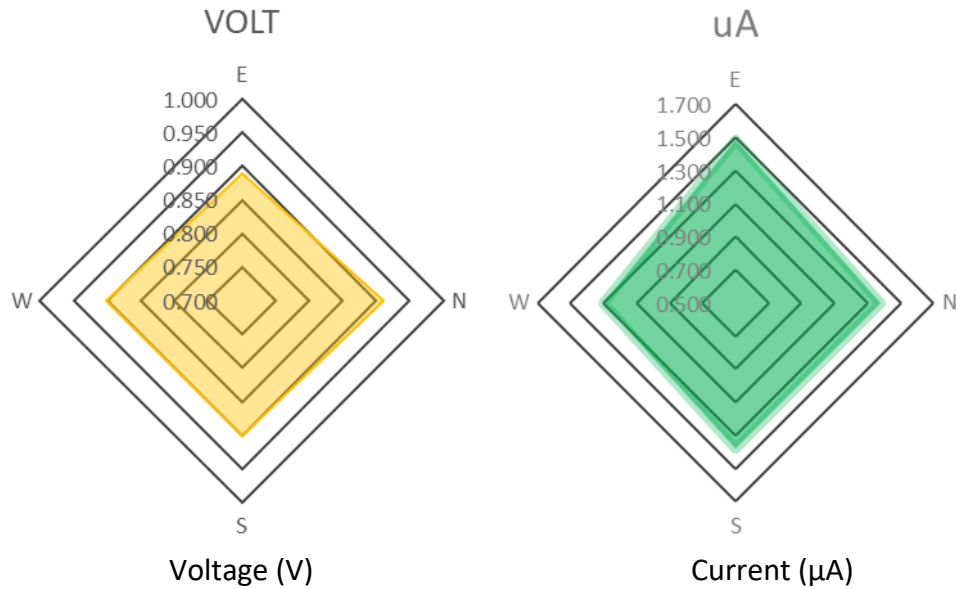
Height (m)	Voltage (V)		ISC ( $\mu$ A)	
	mean	Variance	mean	Variance
0.0-0.5	0.032	0.001	0.471	0.164
0.5-1.0	0.030	0.001	0.427	0.062
1.0-1.5	0.036	0.001	0.441	0.068
1.5-2.0	0.028	0.000	0.263	0.091
2.0-2.5	0.033	0.001	0.218	0.060
2.5-3.0	0.025	0.001	0.332	0.269

Table 4: Statistical mean and variance of bipolar voltage measurements considering height at the tree.

### 3.3 Influence of orientation

Very similar values and no significant differences can be found among the cardinal point placement of the electrodes ( $p$ -value = 0.871), as can be observed in figure 8. However, it is necessary to point out that a tiny significant difference was detected only for ISC between the east and the other cardinal points ( $p$ -value = 0.048). It was decided to continue to obtain data in order to analyse more in detail the possible influence of orientation on ISC. Thus, three other measurements were carried out on

1 21<sup>st</sup> April 5<sup>th</sup> and 12<sup>th</sup> May 2018. None of them showed statistically significant  
 2 differences, neither for voltage nor for ISC, as can be seen in table 5. Consequently,  
 3 these additional measurements ruled out a possible influence of orientation on voltage  
 4 and ISC.



6  
7  
8  
9 Figure 8: Average values for voltage and electrical current in function on the cardinal orientation.

10  
11

	Voltage (V)				ISC (µA)			
	E	N	S	W	E	N	S	W
21/04/2018								
Min	-0,828	-0,864	-0,864	-0,833	-1,830	-1,540	-1,670	-1,430
Max	-1,109	-1,125	-1,109	-1,114	-5,100	-4,760	-4,660	-4,660
Mean	-1,014	-1,033	-1,026	-1,025	-3,411	-3,126	-3,173	-2,860
Variance	-0,007	-0,008	-0,007	-0,006	-0,008	-1,001	-0,874	-1,033
05/05/2018								
Min	-0,713	-0,715	-0,706	-0,703	-0,350	-0,370	-0,030	-0,370
Max	-1,017	-1,032	-0,998	-1,015	-1,470	-1,610	-1,530	-1,610
Mean	-0,876	-0,900	-0,877	-0,886	-0,871	-0,876	-0,817	-0,880
Variance	-0,001	-0,001	-0,001	-0,001	-0,012	-0,011	-0,011	-0,012
12/05/2018								
Min	-0,742	-0,756	-0,747	-0,741	-0,010	0,000	-0,010	-0,010
Max	-0,841	-0,852	-0,852	-0,838	-0,310	-0,310	-0,290	-0,310
Mean	-0,780	-0,802	-0,791	-0,793	-0,167	-0,141	-0,154	-0,152
Variance	-0,012	-0,015	-0,012	-0,012	-0,132	-0,137	-0,173	-0,154

12  
13  
14 Table 5: Statistical mean and variance of voltage measurements referenced to the ground considering orientation at the tree.

1 **3.4 Optimal electrode configuration**

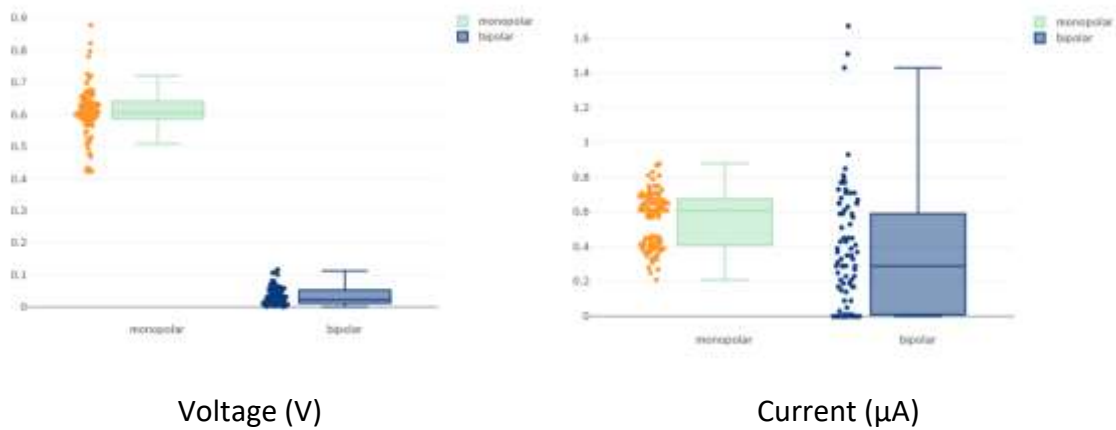
2

3 The results confirm significant differences ( $p$ -value  $< 0.001$ ) in the data obtained  
4 according to electrode configuration, either referenced to the ground or bipolar.  
5 Figure 9 shows this result graphically.

6

7 The obtained results demonstrate that the measured voltage values show a clear and  
8 significant difference between the electrode configurations, with an average value of  
9 0.626 V for the referenced to the ground measurement and a value of 0.036 V for the  
10 bipolar measurement. On the other hand, the observed values for ISC show smaller  
11 differences than for the voltage (see figure 9). Thus, the difference between the means  
12 is much smaller with an average of 0.597  $\mu$ A for the referenced to the ground  
13 measurement and 0.471  $\mu$ A for the bipolar measurement. However, these small  
14 differences are statically significant ( $p < 0.001$ ).

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Figure 9: Significant differences between the bipolar and referenced to the ground configurations in terms of voltage (left) and ISC (right) ( $p$ -value  $< 0.0001$ ).



#### 1 4. DISCUSSION

2  
3 The first result to consider is the observation of a negative electric potential difference  
4 of the tree with respect to the ground. This observation is consistent with the different  
5 authors who have studied the voltage in several tree species [8,10,11,30]. Regarding the  
6 direction of ISC, Le Mouël<sup>[25]</sup> concludes on the presence of positively charged islands  
7 that produce a reversal of current flow. During the fieldwork for this research, we have  
8 not been able to observe any reversal of the current direction. However, as mentioned  
9 in the materials and methods section, the different sets of measurements were made  
10 under meteorological conditions that were as similar as possible.

11  
12 Moreover, the results indicate that the age of Mediterranean pines has a direct  
13 influence on the electrical potential, since young pines present a significantly higher  
14 voltage than mature trees (0.92 V and 0.24 V respectively). Beside from laboratory or  
15 nursery research [11, 31-33], there are research activities that were carried out with trees  
16 in the natural environment, but those activities were performed with a single tree.  
17 Thus, e.g., Cardoso<sup>[27]</sup> analyse the electrical signals of a eucalyptus tree (*Eucalyptus*  
18 *globulus*) with a height of 15 m and a diameter at breast height of 45 cm. Although the  
19 age of the tree was omitted, we concluded that they were done on mature trees  
20 considering the specified tree dimensions. The same happens in other works [10, 26, 28-  
21 30].

22  
23 Additionally, when comparing the voltage values obtained by these authors with ours,  
24 they are close to those obtained with our sample of mature trees. Furthermore, the  
25 results obtained in our research on the electric signal difference between young and  
26 mature trees could be compared with other research related to physiological changes  
27 and age in trees. Thus, for example, Sellin<sup>[63]</sup> observes that the sapwood thickness, in  
28 which the physiological activity is concentrated, decreases its relative proportion when  
29 the trees' age. Also, Rosenthal<sup>[64]</sup> demonstrate that the main functional parameters  
30 for the tree's mineral nutrition decrease as the age of the trees increases, reverting a  
31 behaviour similar to the *Pinus halepensis* volumetric increase functions developed and  
32 published by Montero<sup>[65]</sup>.

33 Furthermore, the results indicate that both voltage and ISC measurements show  
34 uniform distribution patterns in the trunks. Consequently, the measurement does not  
35 vary depending on the height or orientation positioning of the electrode in the trunk.  
36 Le Mouel<sup>[25]</sup> shows a slight tendency to increase the voltage values with increasing  
37 electrode positioning height at the trunk. However, it should be borne in mind that this  
38 research was only carried out on a single tree. Our research cannot verify this  
39 presumed increase either in bipolar or referenced to the ground measurements. Thus,  
40 in the same way that we have been able to demonstrate with the results obtained in

1 our research, Gibert <sup>[10]</sup> and Love <sup>[6]</sup> do not observe significant differences between the  
2 measurement of voltage at different heights of the electrode's position in the trunk  
3 xylem respecting to the ground. Furthermore, our mean voltage values obtained are in  
4 the same order of magnitude as those of the literature consulted <sup>[6, 10]</sup>, despite the fact  
5 that these authors carried out their work outside and under laboratory conditions,  
6 respectively. Thus, in accordance to Gibert <sup>[10]</sup>, we can discard the assumption of the  
7 electrokinetic theory as a generator of the difference in electrical power in trees.  
8 According to the electrokinetic theory, the electric field would be proportional to the  
9 pressure gradient <sup>[67]</sup>, which transferred to a plant, would be the flow of sap per unit  
10 area. Therefore, this theory would imply a linear increase in the amplitude of the  
11 electrical potential with the height in the trunk, at least on average.

12  
13 In the case of the electrode placement depending on the orientation, our voltage  
14 results also coincide with those obtained by other authors <sup>[6,30]</sup>, which were obtained  
15 in measurements on individual trees. The values of current in short-circuit ISC is  
16 presented in only one work <sup>[6]</sup>, instantaneous current in short-circuit ISC values shown  
17 there are quite similar to our registered values. However, the distribution of the ISC is  
18 not mentioned. Since the voltage distribution is homogeneous, we expected the same  
19 with ISC, so we have not arguments against the hypothesis that the distribution of the  
20 ISC should behave differently.

21 In conclusion, the electrical current and voltage do not vary depending on the  
22 measurement height or the orientation of the electrode, which reduces the difficulty  
23 of fieldwork to collect data, expanding the possibilities of installation of measurement  
24 equipment. Furthermore, the use of young trees in ground-referenced measurements  
25 gives the possibility of working with higher value electrical signals. All this will allow us  
26 to optimize the placement of the measurement electrodes in the trees depending on  
27 the circumstances. And it also allows us to consolidate the knowledge of how static  
28 factors influence the measurement of electrical signals.

29 Consequently, this allows us the opportunity to continue researching the dynamic  
30 behaviour of electrical signals on a population of *Pinus halepensis* in a Mediterranean  
31 ecosystem. We plan to evaluate the influence of other factors such as the trees' cycle  
32 variations (daily, monthly and annual) or even the different characteristic  
33 meteorological variables of the Mediterranean climate. The present and future works  
34 will be the basis for the use of measurable electrical signals in trees as a means of  
35 indicating the state of individual trees and forest mass.

36 We work with long term objective of developing low-cost and easy-to-use devices that  
37 could be integrated with other sensing technologies and would allow more accurate  
38 and correct decision-making in forest planning and management through constant  
39 monitoring of trees. .

1 **GEOLOCATION INFORMATION**

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Datum: ETRS89N  
Latitud: 39° 45' 28.80" N  
Longitud: 0° 30' 36.36" W  
Huso UTM: 30  
Coord. X: 713.297,97  
Coord. Y: 4.403.863,10

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4 **DISCLOSURE OF INTEREST**

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6 The authors report no conflict of interest

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