Cold resistance evaluation in different species and provenances of *Juglans* sp.

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INTRODUCTION

Juglans regia is known for its valuable timber. The vulnerability of the southern provenances of this species to low temperatures is one of the main problems to expand its production on the Mediterranean area. According to the climate change estimations on this warm area, the risk period of frost damages and that of summer dry are increasing, this situation could get worse.

The aim of the study is to evaluate different *Juglans* species and provenances (populations) from distinct climatic and agronomic conditions, submitted to an artificial autumn frost. Also, it would be compared different methods for evaluation low temperature effects on this kind of plant material.

MATERIAL AND METHODS

Two assays where carried out: the first one was with three year old clones of four *Juglans* species (Table 1), and the second one, with 8 year old trees of four Spanish provenances (Figure 1), from two planting sites, Reus (Catalonia), with Mediterranean climate and O'Pino (Galicia), Atlantic climate.

The 2nd week of November of 2009, one year old budsticks of both assays were collected. In order to know the vegetative stage of the analyzed trees, a control of the leaf fall in each tree was done, following the description of Díaz (2001), specified in table 2. The level 3 of the leaf fall (where the 50% of the leaf are fall off) is considered as the beginning of the buds inactivity, which indicates the beginning of the tree dormancy.

The leaf fall level, which were the trees in the moment of the budsticks collection, are indicated in table 3 (Juglans species) and the leaf fall level of the two planting site of Spanish provenance analyzed, Reus and O Pino, are indicated in table 4.

TABLE 1. PLANT MATERIALS USED IN THE FIRST ASSAY

Specie	Clone	Origin
J. nigra L	Ng-23	Nord-east USA
J. nigra L	Ng-7	Nord-east USA
J.regia L	MBT-230	Tarragona (Spain)
J.regia L	Po-6	Pontevedra (Spain)
J. major (Torr) A. Heller	Mj-209	USA
Jxintermedia	Ng23×Ra	Híbrid

TABLE 2. STAGES OF LEAF FALL IN JUGLANS, DESCRIBED BY DÍAZ (2001)

Level of leaf fall	Percentage of fallen leafs
1	100%
2	75-99%
3	50-75%
4	25-50%
5	0-25%

TABLE 3. LEAF FALL STAGE DURING THE BUDSTICKS COLLECTION ON *JUGLANS* CLONES (11TH OF NOVEMBER)

Clone	Level of leaf fall
Mj-209	4
Ng-7	3
Ng-23	3
Ng23 x Ra	5
MBT-230	5
MB Po-6	5

TABLE 4. LEAF FALL LEVEL DURING THE BUDSTICKS COLLECTION ON SPANISH PROVENANCES (12TH NOVEMBER), IN THE REUS AND O'PINO PLANTING SITES

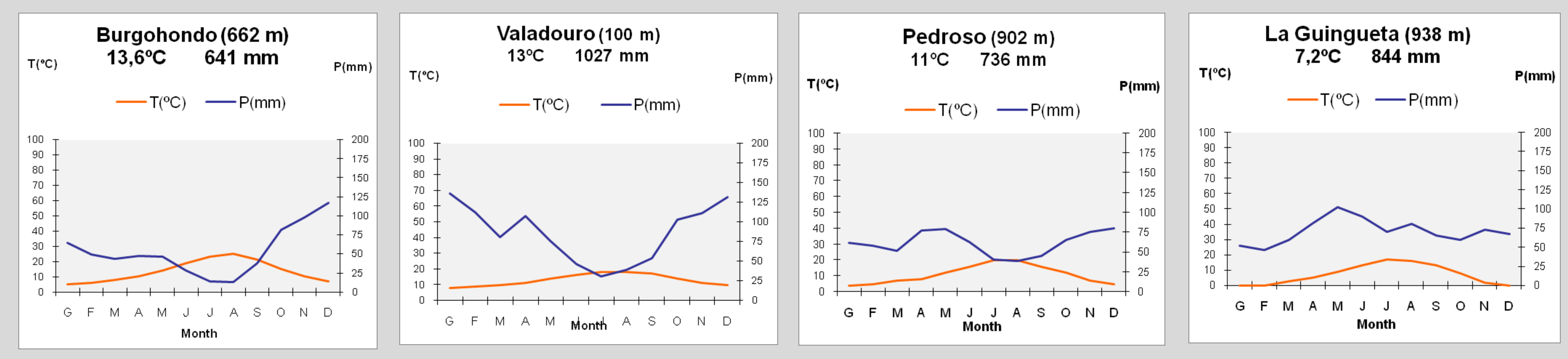
Provenance	Level 5(%)		Level 4(%)		Level 3(%)		Level 2(%)		Level 1(%)	
	RP	OP	RP	OP	RP	OP	RP	OP	RP	OP
Valadouro	0	0	11.5	8.5	56.3	23.5	29.1	35.5	1.1	30
La Guingueta	0	0	7.0	6.2	48.0	16.9	41.0	36.23	4.0	35.74
Pedroso	0	0	10.1	9.5	42.8	13.7	38.5	38.09	7.7	38.09
Burgohondo	0	0	0	9.58	32.0	9.5	53.8	26.94	14.1	56.62

RP and OP means Reus Plantation and O Pino plantation
RP: nuber of Valadouro's trees: 87, La Guingueta: 91, Pedroso: 100 and Burgohondo: 78.
OP: nuber of Valadouro's trees: 200, La Guingueta: 189, Pedroso: 207 and Burgohondo: 219.

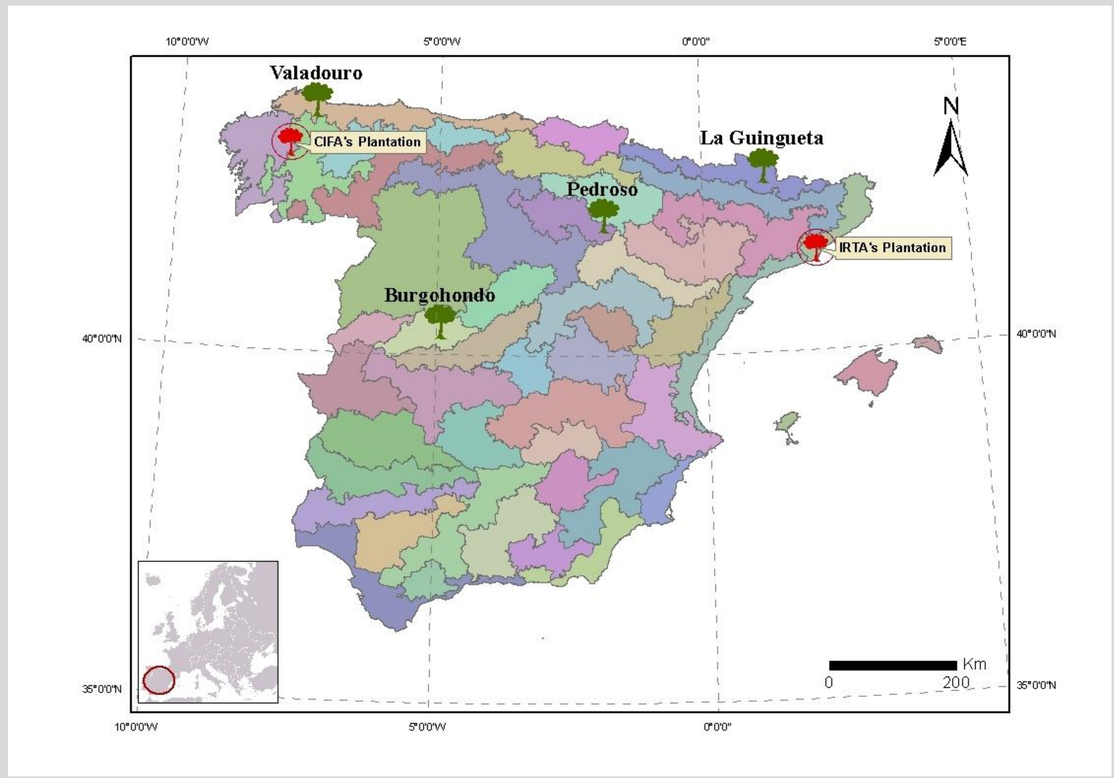
The excised budsticks were distributed by blocks in order to submit them to different temperatures below zero using a programmable freezing chamber. The initial temperature inside the chamber was 5°C, and it was decreasing at 2.5°C/hour to get the temperature according to each treatment.

There was a control treatment, at 5°C, and the freezing temperatures applied were, in the first assay: T1= -3.3°C; T2=-3.7°C; T3=-6.0°C; T4=-8.6°C; T5=-16.5°C. In the second assay the temperatures were: T1= -2.2°C; T2=-3.4°C; T3=-4.3°C; T4=-7.1°C.

FIG 1. CLIMATIC CHARACTERISTICS AND GEOGRAPHIC LOCALIZATION OF THE FOUR PROVENANCES (ASSAY 2) AND THE TWO PLANTING SITE



Source: Atlas Climático Digital de la Península Ibérica



Source: García del Barrio, 2001. Regiones de Identificación y Utilización de Material Forestal de Reproducción

Once the freeze/thaw (at 5°C) cycle was completed, the samples were installed in a greenhouse at 20°C and 90% relative humidity, during two weeks.

Three tests were used in order to know the frost damage occurred in the samples: relative electrolyte leakage (REL), visual scoring (VS) of the cambium and of the apical/lateral buds and chlorophyll fluorescence in cortical bark chlorenchyma. To perform the first test, a couple of slices were cut from each sample, before to place it in the greenhouse, washed with distilled water and put into a separate test tube containing 15 ml of H_2O. After all night at room temperature, and 30 min on shaker, the conductivity of the solution was measured (C1). Then, the tubes were submitted 90 min to 80°C to ensure the completely electrolyte leakage and a second conductivity measure was done (C2). REL (%) was calculated as: REL=C1/C2*100.

After two weeks in the greenhouse, the buds were dissected and the bark was excised from each budstick, in order to evaluate the internal visual damages. The score was done, depending on the damage observed, from 0 (alive tissue) to 100% of damage (completely death). Immediately, chlorophyll fluorescence parameter (Fv/Fm) was measured on the excised cortical bark chlorenchyma, with a Photosynthesis Yield Analyzer (Walz, MINI-PAM).

RESULTS AND DISCUSSION

A significant difference for cold resistance between clones was observed **in relation to** the temperatures applied. The temperature and clone interaction was significant too (Fig. 2). The species *J. nigra,* originated in deciduous forests of the east-USA and Canada (Williams 1990), shows to be the most autumn cold resistant, as it was expected because of its early annual shoots hardening and short vegetative period. On the other hand the Spanish *J. regia* clones were the most affected. These clones are: MBT-230, from Mediterranean climate, and MB Po-6, from Atlantic climate. Both clones of *J. regia*, selected by their growth habit, had longer vegetative period than *J. nigra*. As it was expected the hybrid had intermediate autumn frost resistance, showing always values between its genitors.

A ranking of autumn frost resistance was established from the results: ***J. nigra* ≥ *J. major* > *Jxintermedia* > *J.regia*** (from the most to the less resistant). This range was directly related to the origin of each material.

A regression between the visual scoring and the other tests was done, both laboratory tests, REL & Fv/Fm, had good correlation in the *Juglans* species studied (Table 5). They can be considered good indirect estimators to determinate low temperature resistance.

TABLE 5.REGRESSION BETWEEN VISUAL SCORING (VS) WITH REL TEST, AND VS WITH FV/FM PARAMETER

	REL (%)	Fv/Fm
Visual scoring (VS)	R^2= 0.81***	R^2= 0.71***

**** represent significance level p<0.001*

On the second assay the most relevant differences observed were between the four *J. regia* provenances studied in the test of REL (%): Valadouro provenance was the least cold resistant. In the test of chlorophyll fluorescence, a significant interaction between planting site and provenance existed. In O Pino (Atlantic coast) all the provenances had the same behavior, while in Reus (Mediterranean climate) Valadouro provenance showed a major susceptibility to low temperature than others. The differences in leaf fall between planting sites could be an explanation of that result. The advanced senescence of Valadouro in O Pino could be a protection against autumn low temperatures (table 4).

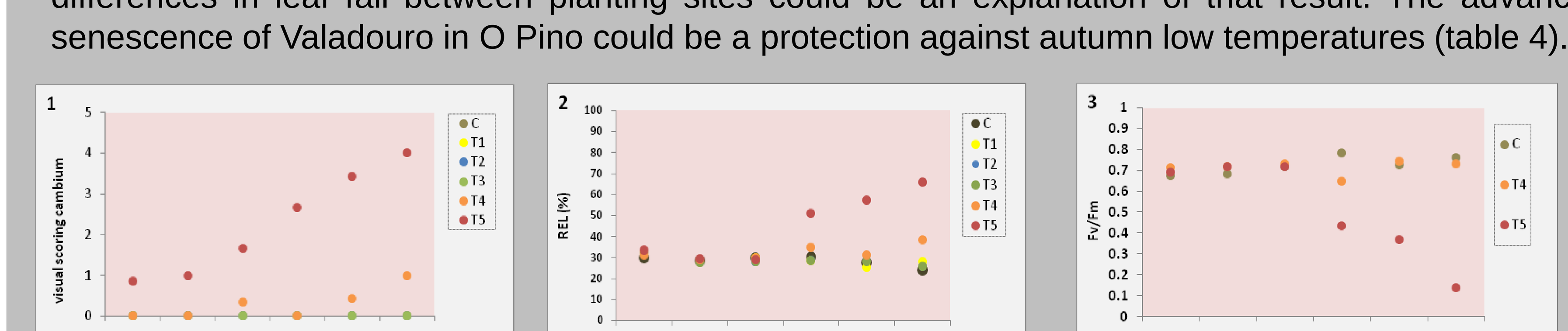


FIG 2. THE INTERACTION BETWEEN THE CLONES ANALYZED AND TEMPERATURE TREATMENTS IS REPRESENTED WITH THREE DIFFERENT VARIABLES: **1.** VISUAL SCORING OF THE DAMAGE; **2.** RELATIVE ELECTROLYTE LEAKAGE (%); **3.** FV/FM.

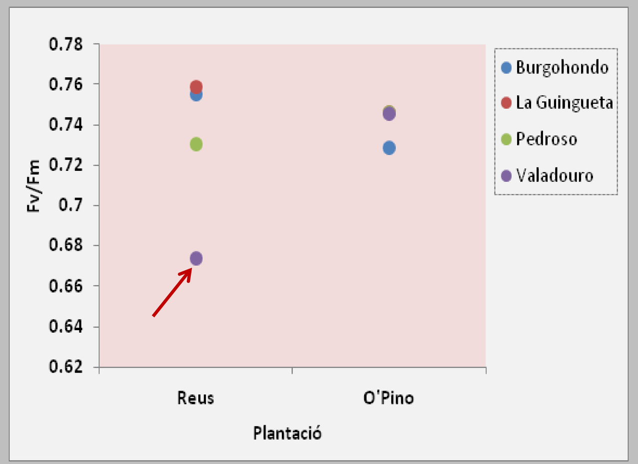


FIG 3. INTERACTION BETWEEN THE PROVENANCES AND THE PLANTATION SITE. RED ARROW SHOWS SIGNIFICANT DIFFERENCES BETWEEN VALADOURO PROVENANCE IN REUS PLANTATION SITE.

CONCLUSIONS

❑ The expected ranking of autumn frost resistance had been observed between the different species of *Juglans* evaluated. The hybrid show an intermediate autumn frost resistance between the parents.

❑ Autumn frost damages of Spanish provenances iseems to be related to the planting site. New analysis must be done in this sense.

❑ The Fv/Fm parameter of the cortical bark chlorenchyma is a good autumn frost damages estimator. Its determination is an easy and fast, but more analysis must be done to consider Fv/Fm as a main test to determinate frost resistance.

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