



# Comparative analysis of different methods for evaluating evergreen oaks mycorrhized with black truffle

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## 0. Introducción.

### Methods for the evaluation of mycorrhized plants

#### Forestry quality of the plants

Real Decreto 1220/2011, de 5 de septiembre,



Main methods used for plant certification in Spain and in Europe

Método de Reyna et al. CEAM-Valencia (España, 1997)

Método de Chevalier et al. INRA-ANVAR (Francia, 1972)  
Método de Palazón et al. INIA – Aragón (España, 1999)

Método de Bencivenga et al. Universidad de Perugia (Italia) (1987 y 1995)

Método de Fischer y Colinas. Universidad de Lleida (España, 1996)



## 1. Objectives

Evaluate the main methodologies used for assessing mycorrhization in plants produced in nurseries

Establish the correlation existing between the different methods

## 2.Experimental Desing.

### 1st. Part: Sampling of mycorrhized plants

- Choosing the nurseries producers of mycorrhized plants
- Sampling of **120 plants** was carried out the 18th. of November 2011



Nursery1:  
12 plants/batch (5 batchs) = 60 plants



Nursery 2:  
12 plants/batch (5 batchs) = 60 plants



## 2.Experimental Desing.

### 1st. Part: Sampling of mycorrhized plants

*Processing of the plants*

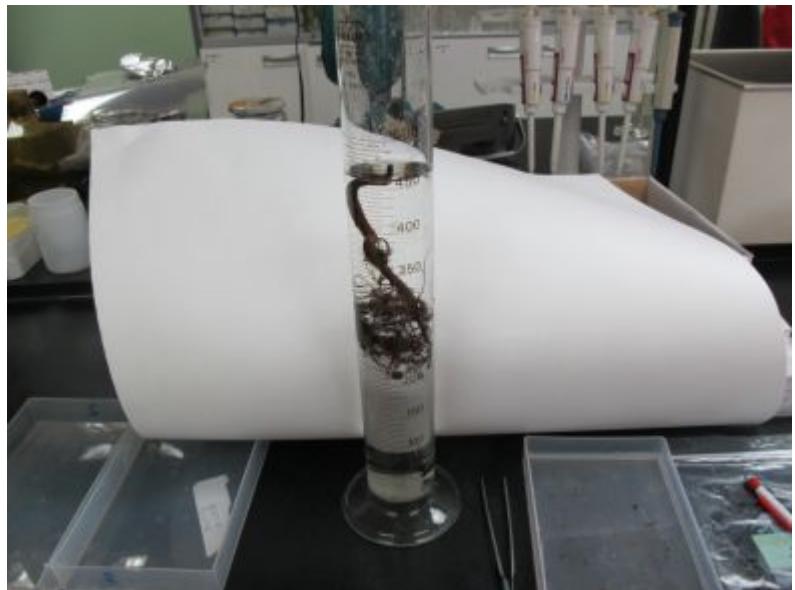
- ✓ 1<sup>st</sup> Washing
- ✓ Labeling of each plant
- ✓ Measurement Root system Length , Diameter of the stem
- ✓ Bagging of each root system
- ✓ Storage at -20°C



## 2.Experimental Desing.

2<sup>nd</sup>. Part: Evaluation of each batch using all 5 methods (same experimenter)

- ✓ Thawing of batches
- ✓ Gentle washing using ultrasound bath
- ✓ Measurement of the root system volume of each plant

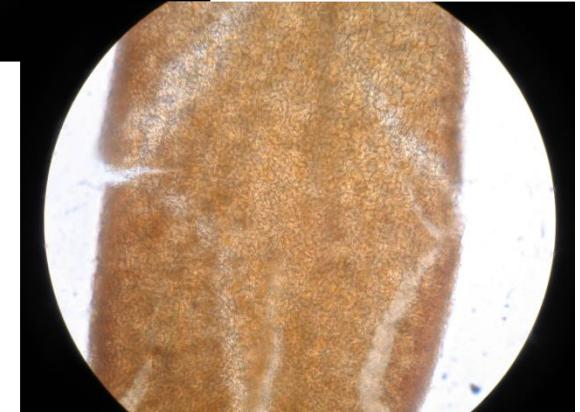


## 2.Diseño experimental.

**2<sup>nd</sup>. Part: Evaluation of each batch using all 5 methods (same experimenter)**

First observation of the plant

- ✓ Evaluation of the forestry quality
- ✓ -Microscopic observation of the presence/absence of mycorrhized roots with T.m. and/or other different fungi not used in the inoculation process.



## 2.Diseño experimental.

### 2<sup>nd</sup>. Part: Evaluation of each batch using all 5 methods (same experimenter)

Evaluation of the plant: starting with the less destructive method and ending with the most destructive

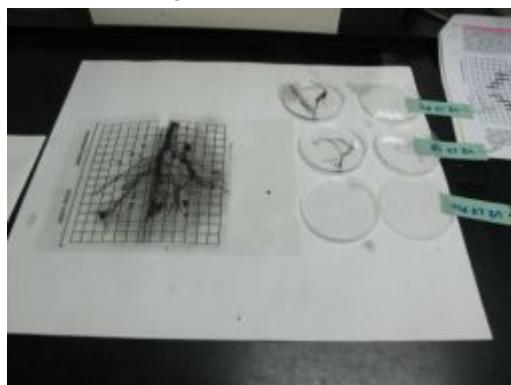
1ºReyna et al. CEAM-Valencia



2ºChevalier et al. INRA-ANVAR



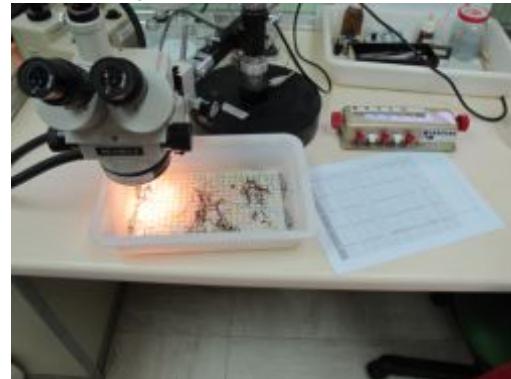
3ºBencivenga et al. Universidad de Peruggia



4ºPalazón et al. INIA – Aragón



5ºFischer y Colinas. Universidad de Lleida



### 3.Resultados y discusión.

Do we have the same evaluation results across all methods?

Assessment per batches

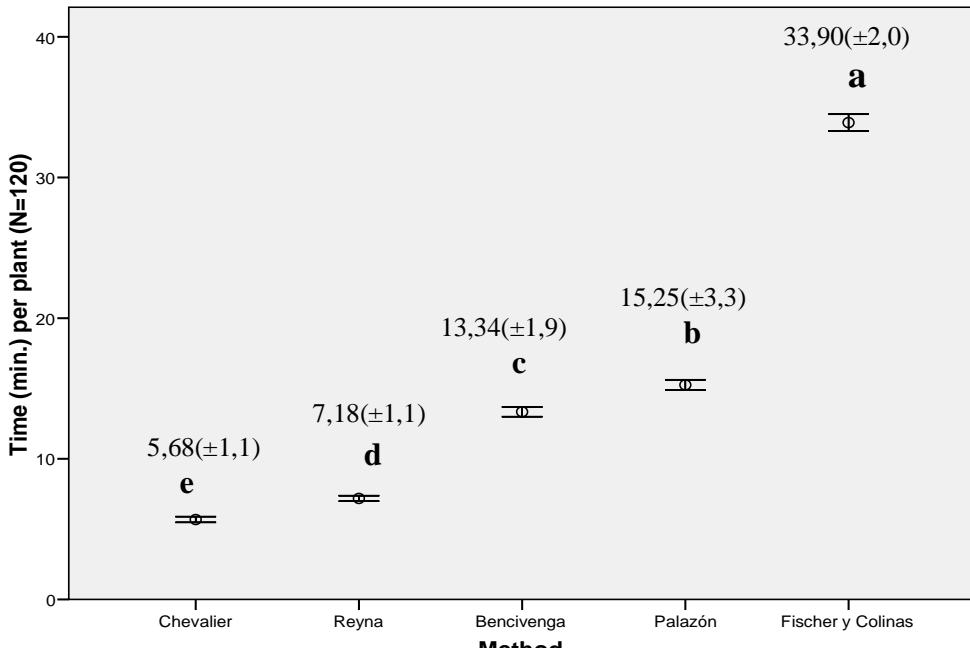
Lote	%	Reyna et al	Chevalier et al.	Bencivenga et al.	Palazon et al.	Fisher and Colinas
1	42.3	si	si	si	si	si
2	31.2	no	si	si	si	si
3	31.2	no	si	no	no	si
4	33.5	si	si	no	si	si
5	30.0	si	si	no	no	si
6	25.2	si	si	no	no	no
7	18.9	no	no	no	no	no
8	33.9	si	si	si	si	si
9	25.3	no	si	no	no	si
10	32.4	si	si	no	no	si

6 9 3 4 8

$\chi^2: 10.833$  p: 0.029

### 3. Results and discussion.

#### Correlation between sampling time per plant and each method



$\chi^2=538.814$        $p=0,000$       Error Bars: 95% CI

### 3.Resultados y discusión.

#### Correlation between methods with respect to the % of mycorrhization

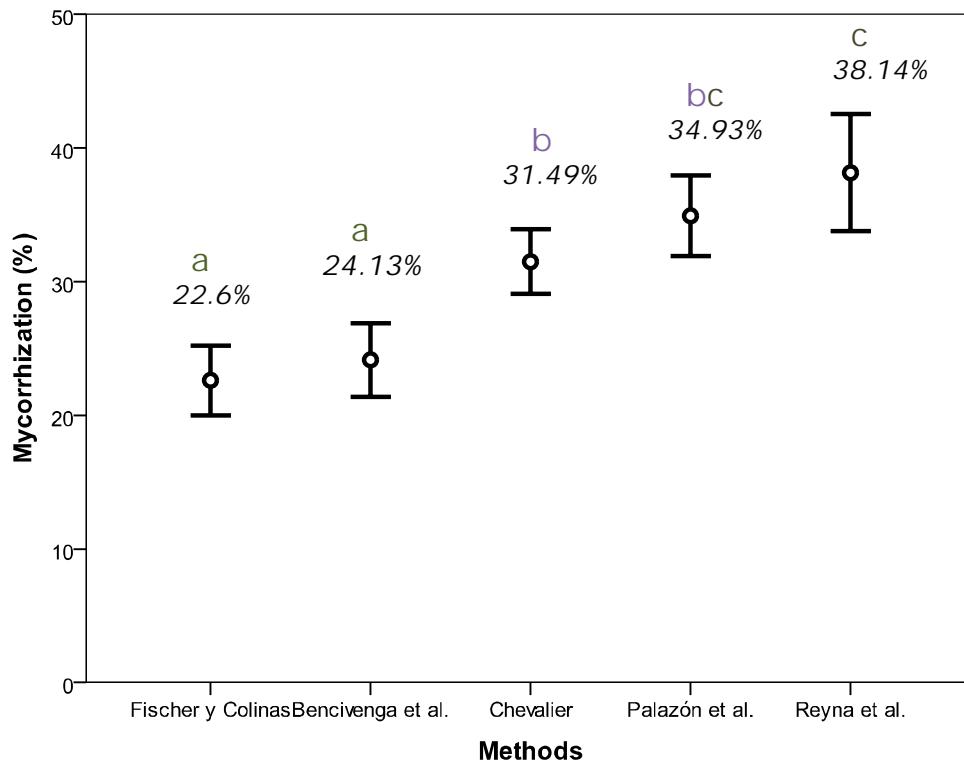
			Reyna	Chevalier	Bencivenga	Palazón	Fischer y Colinas
Rho de Spearman	Reyna	Coeficiente de correlación	<b>1.000</b>	.119	.184	.101	.159
		Sig. (bilateral)	.	.219	.057	.313	.106
		N	109	109	107	101	105
	Chevalier	Coeficiente de correlación			<b>0.397(**)</b>	<b>0.523(**)</b>	<b>0.519(**)</b>
		Sig. (bilateral)			.000	.000	.000
		N			117	110	115
	Bencivenga	Coeficiente de correlación				<b>0.494(**)</b>	<b>0.574(**)</b>
		Sig. (bilateral)				.000	.000
		N				109	114
	Palazón	Coeficiente de correlación					<b>0.609(**)</b>
		Sig. (bilateral)					.000
		N					110
	Fischer y Colinas	Coeficiente de correlación					<b>1.000</b>
		Sig. (bilateral)					
		N					115

\*\* La correlación es significativa al nivel 0,01 (bilateral).

### 3.Resultados y discusión.

Correlation between methods with respect to the % of mycorrhization?

Graphic representation of the percentage confidence interval ( $\alpha = 0.05$ ) mycorrhization obtained through various methods.



All methods tend to overestimate the percentage (Gógán 2011)

## 4. Conclusions.

- The methods based on Fischer and Colinas, and Bencivenga et al. gave lower % of mycorrhization for all batches
- The method based on Reyna et al. gave the highest mycorrhization level and didn't correlate with any of the other methods. All remaining 4 methods did have some correlation in their assessment of the mycorrhization level
- All 5 methods gave the same results for the extreme cases of high level and absence of mycorrhization.
- These results highlight the necessity for unified evaluation criteria of mycorrhized plants

# Thank you for your attention

## Acknowledgments



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