

INOCULATION OF HAZELNUT GROVES WITH *Tuber brumale* & *Tuber melanosporum* Vitt.

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ABSTRACT

The hazelnut tree has a great social and economic value in the NE of Spain, nowadays sunk in economic crisis. 15000 Hectares of these groves lay on potential truffle producing areas. *Tuber melanosporum* and *Tuber brumale* fruit in some of them naturally. Our aim is to develop inoculation techniques in mature groves and later to coordinate the truffle and hazelnut cultivation, trying to get an added value to the traditional harvest. In this work, large inoculations have been carried out in mature hazels with *Tuber brumale* on 3230 hazels, and *Tuber melanosporum* on 1300 hazels, in a total area of 11,3 hectares. They were carried out two inoculations with spore inoculum in all the trial fields, spring-fall within the same year or spring-spring with one year delay. Percentage of trees that got mycorrhizae of *Tuber melanosporum* one year after the first inoculation are between 28,6% and 45,2% of the inoculated hazels. The hazels that present mycorrhizae of *Tuber brumale* are between 24,1% and 56,2% after the first inoculation.

INTRODUCTION

Hazelnut production in Spain is nowadays sunk in an economic crisis, because of the prizes and imports especially from Turkey. In Catalonia (North East of Spain) there are 18.537 hazelnut groves (*Coryllus avellana*) (1999 Census¹), most of them on truffle potential areas. *Tuber melanosporum* and *Tuber brumale* fruits on them naturally. In 1995 we start the present work with the aim to develop methods to inoculate those nature hazel groves with truffle, in order to get an added value to the traditional hazel crop.



● Distribution of *Tuber melanosporum* in Spain

We found that the production and presence of mycorrhizae of *Tuber* and other fungi in these groves are scarce, probably due to the use of pesticides and fertilizers.

The hazel tree has a high number of shallow fine roots, so it has been easy to inoculate and check the mycorrhizae later.

Field inoculation from spores or soil from truffle producing areas is quite old, with some good results 1-2 years later, although is complicated to know if the fruiting comes from the soil plough or from the inoculation. From mycorrhized seedling outplanted on field, the truffle infection can develop and infect new neighbouring plants². Reinoculation directly in the field of *Tuber uncinatum* already mycorrhized plants one year after outplanting, improves Bourgundy truffle production. The inoculation with *Tuber uncinatum* of 14 years old trees that just produce *Tuber brumale*, leads 5 years later to a production of the *Tuber uncinatum* in the reinoculated areas^{3,4}. Reyna⁵, with spore inoculation on mature Holm oaks, got truffle mycorrhizae on 10 of the 17 samples. Lo Blue⁶ studied the inoculation with root fragments on nature trees. Tanfulli et al.⁷ inoculated *Quercus pubescens* with *Tuber melanosporum* and *Tuber aestivum* at the outplanting time. Two years later the whole root system had high levels of mycorrhizae from the inoculated truffles, with few other fungi.

Previous works inoculating mature hazels

We started studying the truffle ecology on hazel groves where truffles fruits naturally

Tuber melanosporum, fruits on those hazels at higher elevations, where watering is not possible. Those trees are smaller; never reach a full canopy with poor weeds. Soil has a higher pH and with less organic matter.



The first inoculation trials were done by inspection of spore inoculum.

Our aim was to develop an agronomical way of inoculation, in order to be cheap, easy and fast. Inoculations with tractor gave the following results.

TRIAL FIELDS					
Trial fields	Number of trees	Age	Elevation	Orientation	Mycorrhization 2 years later
A	110	35	950	SW	55%
B	40	17	908	S	69%
C	235	24	995	W	50%

TRUFFLE PRODUCTION			
Trial fields	First year	Second year	Third year
A	400g (3 hazels)	580g (3 hazels)	150g (2 hazels)
B	30 g (1 hazel)	450g (5 hazels)	310g (6 hazels)
C	No production Mycorrhization level 4 years later: 73%		

MATERIAL AND METHODS

In this study, following previous results, we have inoculated 1300 hazelnuts with *Tuber melanosporum* and 3230 with *Tuber brumale*. We choose five trial sites for *T. melanosporum* and 11 trial sites for *T. brumale* from the Prades Range, located 120 km southwest from Barcelona. Before any inoculation, a root sample was taken from 10% of all the trees to check the initial level of mycorrhizae of each truffle, in order to evaluate some raise after inoculations. Roots were taken in October-November and stored with FAA until their observation at microscope.

Inocula was made from nature truffles, harvested in the Prades Range, at the end of truffle season (February-march). They were cleaned, surface sterilized, microscope checked for specie determination and stored frozen. All the inoculations have been done two times.

Truffles are blundered and spore concentration counted at Neubauer Chamber. Inocula are mixed with hydrogels, root promoting factors and spore germination promoting factors (process under patent)

Inoculation were done by tractor, ploughing at 10-20 cm deep, breaking fine rots, usually at 1,5 m from the tree trunk. Two systems were used: a simple one with a 50L bottle over a plough, with a tap at the bottom and two hoses tied at each side of the ploughing machine. As tractors runs forward, inoculum drops by gravity and get buried into the soil at a dose of 0,5 L/tree. The second machine was a large bottle of 600L with an air pump that allows us to adjust pressure inside the tank, adjusting at the same time the inoculation dose at 1L/tree. The time of inoculation is reduced to 1h/Ha/500 trees.

Six months after each inoculation, roots are sampled at a level of 10% of inoculated trees, stored in FAA and checked at microscope for a qualitative analysis: we just checked presence/absence of the inoculated truffle, according with the other works^{8,9,10,11}.

RESULTS AND DISCUSSION

We present the mycorrhizae level six months after the first inoculation. Second inoculation was done on spring 2007 and final result will be at the end of 2007. At this point we still have no data about if it's better to repeat inoculations next autumn or spring.

We have proved that disinfection pre-treatment decrease the level of some mycorrhizal fungi before the inoculations, but they seem to be not necessary as the disinfected trial fields get the same levels of truffle infections as non disinfected fields.

Trial fields inoculated with *Tuber brumale*

Trial Fields	Number of hazels	Age	Elevation	pH
B	240	26	810	7,64
C	400	23	580	7,66
D	150	29	1005	7,76
E	150	29	995	7,49
F	400	27	90	7,15
G	225	17	700	7,51
H	450	18	890	7,43
I	175	15	595	7,18
J	275	24	750	7,78
K	400	20	620	7,78

Trial Fields	Disinfection pretreatment	Mycorrhizae level before inoculation (%)	Mycorrhizae level after first inoculation (%)
B	YES	17,24	53,84
C	YES	12	45,6
D	NO	11,1	27,6
E	NO	0	42,3
F	NO	0	32,7
G	NO	10	50
H	NO	10	42
I	NO	17,6	24,1
J	NO	12,9	40
K	NO	13,8	56,2

Trial fields inoculated with *Tuber melanosporum*

Trial Fields	Number of hazels	Age	Elevation	pH
L	50	26	810	7,64
M	150	18	890	7,43
N	550	6	700	7,87
O	75	15	595	7,18
P	455	24	750	7,78

Trial Fields	Disinfection pretreatment	Mycorrhizae level before inoculation (%)	Mycorrhizae level after first inoculation (%)
L	YES	17,24	42,8
M	NO	10	No data
N	NO	7,1	45,2
O	NO	17,6	28,6
P	NO	No data	40

Similar results were found by our team in previous tests¹² and Frochot et al. ¹³.

All trial fields had an increase in the level of truffle mycorrhizae six months after spring, despite there was no rain at all during 4 months after inoculations. Traditionally hazel groves have been planted at densities 5x5 m, 6x4 m or 6x6 m, the same we use for black truffle culture. Some of these hazel groves produce truffles naturally despite the higher levels of fertilizer (N:P:K 13:13:15) at 125 Kg/Ha, nitrofosca and several phytosanitary treatments against hazelnut plagues. Hazelnut harvest is made with heavy machines during September-October, fallen leaves are swallowed and powdered back to soil. Usually leaves and branches are burnt and the ashes are spread. Ashes can raise pH and all decreases fresh organic matter levels, that could improve truffle fruiting^{14,15}. More studies are being carried out with the aim to coordinate hazel and truffle production.



Spots where inoculated truffles were harvested

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