

# OPEN FIELD INOCULATION OF ADULT HAZELNUT GROVES WITH *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. 15.000 hectares of these groves lays 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 hazel trees with *Tuber brumale* on 3.230 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 mycorrhizas of *Tuber melanosporum* one year after the first inoculation are between 28,6% and 45,2% of the inoculated hazels. The hazels that present mycorrhizas 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, specially from Turkey. In Catalonia (North east of Spain) there are 18.537 hazelnut groves (*Corylus avellana*) (1999 Census), most of them on truffle potential areas. *Tuber melanosporum* and *Tuber brumale* fruits on them naturally. In 1995 we started 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.

We found that the production and presence of mycorrhizae of *Tuber* and other fungi in these groves were scarce, due to the use of pesticides and fertilizers. Hazels have a high number of shallow fine roots; so it has been easy to inoculate and check mycorrhizae later.

Field inoculation from spores or soil from truffle producing areas is quite old, with some good results one or two years later, although it's complicated to know if the fruiting comes from the soil plough or from the inoculation. From mycorrhized seedlings outplanted on field, the truffle infection can develop and infect new neighbouring plants. Inoculation directly in the field of *Tuber uncinatum* already mycorrhized plants one year after outplanting, improves Burgundy truffle production. The inoculation with *T. uncinatum* of 14 years old trees that produce *Tuber brumale*, leads 5 years later to a production of the *T. uncinatum* in the re-inoculated areas<sup>1,4</sup>. Reyna<sup>3</sup>, with spore inoculation on mature homoaks, got mycorrhizae of truffle on 10 of 17 samples. Lo Blue<sup>6</sup> studied the inoculation with root fragments on nature trees. Tanfulli et al.<sup>7</sup> 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 almost no weed. Soil has a higher pH and with less organic matter.



The first inoculation trials were done by injection of spore inoculum. Between 38% and 72% of the inoculated hazels had some mycorrhiza of the inoculated *Tuber melanosporum*. 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 FIELD				BLACK TRUFFLE PRODUCTION			
Number of trees	Age	Elevation	Orientation	Mycorrhization 2 years later	First year	Second year	Third year
110	35	950	SW	55%	400g (3 hazels)	580g (3 hazels)	150g (2 hazels)
40	17	908	S	69%	30 g (1 hazel)	450g (5 hazels)	310g (6 hazels)
235	24	995	W	50%	Mycorrhization level 4 years later: 73%		

## MATERIAL & METHODS

Following previous results, we have inoculated 1300 mature hazels with *Tuber melanosporum* and 3230 with *Tuber brumale*. We choose five trial sites for *Tuber melanosporum* and 11 trial sites for *Tuber 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. Inoculum 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 twice. Truffles are blander 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 roots, usually at 1,5 m from the trunk. Two systems were used:



Tractor with a large bottle of 600 L with an air pump that allows us to adjust pressure inside the tank. We can adjust at the same time the inoculation dose at 1L/tree. The time of inoculation is reduced to 1hour/Ha/500 trees



Simple system using a 50 L bottle over a plough, with a tap at the bottom and two hoses tied at each side of the ploughing machine. As tractor runs forward, inoculum drops by gravity and get buried into the soil at a dose of 0,5 L/tree.

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 presence/absence of the inoculated truffle, according with the works of Agerer<sup>8</sup>, Etayo & de Miguel<sup>9</sup>, Saez & de Miguel<sup>10</sup> and Verhac<sup>11</sup>.

## 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.

Trial fields	Number of hazels	Age	Elevation	pH	Disinfection pretreatment	Mycorrhizae level before inoculation (%)	Mycorrhizae level after first inoculation (%)
B	240	26	810	7.64	YES	17.24	53.84
C	400	23	580	7.66	YES	12	45.6
D	150	29	1005	7.76	NO	11.1	27.6
E	150	29	995	7.49	NO	0	42.3
F	400	27	970	7.15	NO	0	32.7
G	225	17	700	7.51	NO	10	50
H	450	18	890	7.43	NO	10	42
I	175	15	595	7.18	NO	17.6	24.1
J	275	24	750	7.78	NO	12.9	40
K	400	20	620	7.78	NO	13.8	56.2

Chart 1. Trial fields inoculated with *Tuber brumale*

Trial fields	Number of hazels	Age	Elevation	pH	Disinfection pretreatment	Mycorrhizae level before inoculation (%)	Mycorrhizae level after first inoculation (%)
L	50	26	810	7.64	YES	17.24	42.8
M	150	18	890	7.43	NO	10	No data
N	550	6	700	7.87	NO	7.1	45.2
O	75	15	595	7.18	NO	17.6	28.6
P	455	24	750	7.78	NO	No data	40

Chart 2. Trial fields inoculated with *Tuber melanosporum*

We have proved that disinfection pretreatments 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.

Similar results were found by our team in previous tests<sup>12</sup> and Fréchet et al.<sup>13</sup>. All trial fields had an increase in the level of truffle mycorrhizae six months after spring inoculations, 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 125Kg/Ha, nitrofosca and several phytosanitary treatments against hazelnut plagues. Hazelnut harvest is made with heavy machines during September-October, fallen leaves are wallowed 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<sup>14,15</sup>. More studies are being carried out with the aim to coordinate hazel and truffle production.



Spots where inoculated truffles were harvested



Root sample from soil. New formed black truffle mycorrhiza through the hydrogel.