INOCULATION OF HAZELNUT GROVES WITH TUBER BRUMALE & TUBER MELANOSPORUM VITT.

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ABSTRACT

The hazelnut tree has a great social an 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. We studied the ecology and soil parameters on hazel truffieres. No significative differences were found on the fine and coarse oxidable organic materia between *T. melanosporum* and *T. brumale* soils. 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 crop. In this work, large inoculations using the *Mycoforest Technology®* 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 sporal 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. In both cases, two years later infection degree raised.

KEY WORDS: *Tuber, Corylus avellana,* truffles, field inoculation, *Mycoforest Technology®.*

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*) (CENS AGRARI 1999), 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.

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 plants (CHEVALIER & GRENTE 1978).

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 (CHEVALIER *et al*. 2002); (FROCHOT *et al.* 1999).

Reyna, with sporal inoculation on mature Holm oaks, got truffle mycorrhizae on 10 of the 17 samples (REYNA *et al*. 2002). Lo Blue studied the inoculation with root fragments on nature trees (LO BLUE *et al*. 1990). On 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 (TANFULLI *et al.* 1997).

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 and never reach a full canopy with almost no weeds. Soil has a higher pH and with less organic matter (Fig. 1.).

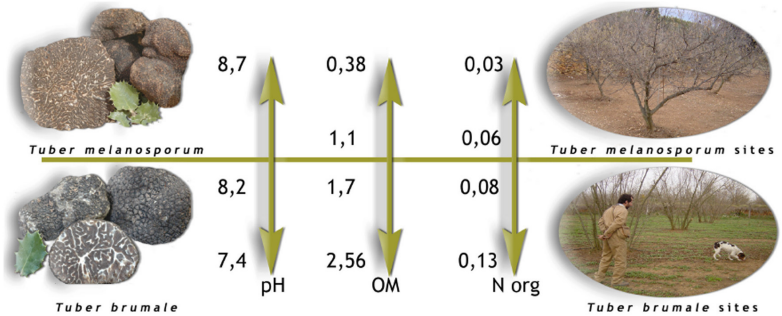

Fig. 1. Soil parameters on natural hazel groves where truffles grow naturaly

Some studies carried in France (RICARD 2003) show differences between oxidable organic materia on *T. melanosporum* and *T. brumale* truffieres, although they come from just four soil samples. Those differences were focused on a higher values of >50µm fraction on *T. brumale* than in *T. melanosporum*.
Our aim was to develop an agronomical way of inoculation, in order to be cheap, easy and fast. Inoculations with tractor gave the results showed in Tab.1 (MORCILLO *et al.* 2007).

TRIAL FIELDS

Trial field	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 %

BLACK TRUFFLE PRODUCTION

Trial field	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	0 g*	0 g*	0g*

*Mycorrhization level four years later: 73%

Tab.1. First inoculated fields and first truffle productions 1, 2 and 3 years after the inoculations.

MATERIAL AND METHODS

We did soil analysis for fine (<50µm) and coarse (50-200µm) organic materia on *Tuber melanosporum* and *Tuber brumale* soils under hazels in order to compare with the previous results obtained at the french studies (RICARD 2003).

In this study, following previous results, we have inoculated 1300 adult hazels 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, Spain (Fig.2). Before any inoculation, a root sample was taken from 10% of all the trees to check the initial level of mycorrhizae of each truffle, as a control. Roots were taken in October-November and stored with FAA until their observation at microscope.

Fig. 2. Study area in NE Spain

Inocula was made from nature truffles, harvested in the Prades Range, at the end of truffle season (February-march). Inocula is mixed with hydrogels, root promoting factors and spore germination promoting factors (following the *Mycoforest Technology®* method).

Inoculations were done by tractor, usually at 1-1,5 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 tractor runs forward, inoculum drops by gravity and get buried into the soil. 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. With this system 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. Root samples are taken at 1-1.5m from the trunk of the tree at both sides of the tree, at the same place where they were inoculated. Roots are stored in FAA and checked at microscope for a qualitative analysis: we just checked presence/absence of the inoculated truffle, according with previous studies (AGERE 1987-1998); (EAYO & DE MIGUEL 1998); (SAEZ & DE MIGUEL 1995); (VERLHAC 1990).

RESULTS AND DISCUSSION

Soil analysis results for fine (<50µm) and coarse (50-200µm) organic materia on *Tuber melanosporum* and *Tuber brumale* under hazels showed no significative difference nor on the <50µm neither on 50-200µm between both truffles (Tab.2).

Tuber melanosporum			*Tuber brumale*			
< 50µm	50-200µm	ratio	< 50µm	50-200µm	ratio	
4,6	5,59	1,22	**3,19**	3,6	1,13	
1,58	1,33	0,84	**1,84**	1,27	0,69	
2,48	3,13	1,26	**4,04**	3,89	0,96	
1,9	2,16	1,14	**3,36**	3,51	1,04	
6,21	5,44	0,88	**6,72**	8,23	1,22	
2,51	2,6	1,04	**7,63**	7,02	0,92	
2,93	3,47	1,18	**1,75**	1,65	0,94	
1,42	1,97	1,39	**3,35**	3,46	1,03	
0,83	0,94	1,13	**3,7**	6,02	1,63	
4,97	4,88	0,98				
2,94	3,15	1,11	**3,95**	4,29	1,06	avarage
1,75	1,67	0,17	**2,00**	2,35	0,26	S.D.

Tab. 2. Oxidable organic materia for fine (<50µm) and coarse (50-200µm) on *Tuber melanosporum* soils (left) and on *Tuber brumale* soils (right) under hazels. (values on %)

These results need further study as they can be usefull for the later management on hazelnut groves infected with truffles.

We present the mycorrhizae level six months after the first inoculation (done on september-october 2006). Second inoculation was done on spring 2007 and hazels were sampled again on november 2008 (1,5 years after the second inoculation). Between 7.1% and 17.6% of the hazel trees have naturally (without any artificial inoculation) mycorrhizas of *Tuber melanosporum* and between 0 and 17.6% have *T. brumale* naturally in the studied area.

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. Similar results were found by our team in previous tests (MORCILLO *et al*. 2003); (FROCHOT *et al*. 1990).

All trial fields had an increase in the level of truffle mycorrhizae after the first and second inoculation, despite there was no rain at all during 4 months after inoculations (tab. 3 & 4).

Trial Fields	Number Hazels	Age	Elevation (m.o.s.l)	pH	DP[1]	MLBFI[2]	MLAFI[3]	MLASI[4]
B	240	26	810	7,64	YES	17,24	53,8	35,7
C	400	23	580	7,66	YES	12	45,6	42
D	150	29	1005	7,76	NO	11,1	48,3	78
E	150	29	995	7.49	NO	0	42,3	ND*
F	400	27	90	7,15	NO	0	32,7	35,7
G	225	17	700	7.51	NO	10	50	35,3
H	450	18	890	7.43	NO	10	42	40
I	175	15	595	7,18	NO	17,6	24,1	38,9
J	275	24	750	7.78	NO	12,9	40	28,6
K	400	20	620	7.78	NO	13,8	56,2	50

[1] DP: Disinfection pretreatment
[2] MLBFI: % Mycorrhizae level before first inoculation
[3] MLAFI: % Mycorrhizae level after first inoculation
[4] MLASI: % Mycorrhizae level after second inoculation
* ND: No data

Tab. 3. Trial Fields inoculated with *Tuber brumale*

Trial Fields	Number hazels	Age	Elevation (m.o.s.l.)	pH	DP[1]	MLBFI[2]	MLAFI[3]	MLASI[4]
L	50	26	810	7,64	YES	17,24	42,8	35,7
M	150	18	890	7,43	NO	10	ND*	33
N	550	6	700	7,87	NO	7,1	45,2	44,8
O	75	15	595	7,18	NO	17,6	28,6	38,9
P	455	24	750	7,78	NO	ND*	40	69,4

[1] DP: Disinfection pretreatment
[2] MLBFI: % Mycorrhizae level before first inoculation
[3] MLAFI: % Mycorrhizae level after first inoculation
[4] MLASI: % Mycorrhizae level after second inoculation
* ND: No data

Tab. 4. Trial fields inoculated with *Tuber melanosporum*

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, and between 33% and 69,4% after the second inoculoation. The hazels that present mycorrhizae of *Tuber brumale* are between 24,1% and 56,2% after the first inoculation, and between 28,6% and 78% after the second inoculation.

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 ould improve truffle fruiting. More studies are being carried out with the aim to coordinate hazel and truffle production.

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