Improving productivity and sustainability of black truffle plantations by microbiological handling of the rhizosphere

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Black truffle production is an expanding crop in Catalonia with high economical potential, especially in agricultural areas with low productivity. In this sense, black truffle cultivation is often developed on poor soils, with low production yields and where trees present nutritional deficiencies and serious phytosanitary problems.
Another problem to be solved in trucficulture is crop irregularity, possibly due to suboptimal production conditions, both in the nursery and in the field. As a major diference from the cultivation of trees for biomass or for the cultivation of fruits, in this case we work with a much more complex interaction between the tree and the rhizosphere or interface zone between the root of the plant and the soil. However, the increase of monospecific plantations (generally holm oaks) can cause an increase in certain diseases and pests that decrease the production of truffles. Management of the rhizosphere can contribute to the general improvement of plant vigor and its tolerance to biotic factors without the need to use phytosanitary products.
In this project we evaluated the capacity of different organic substances and rhizobacteria, some isolated from wild truffieres described by Vilanova et al (2013) with the intention of improving the biotic and abiotic conditions of the rhizosphere, considering the presence and availability of nutrients, Development of the vegetative phase of truffle mycelium, vigorousness of the tree (nutritional status) and control of pathogens.
The follow-up of the fungus response will be carried out in collaboration with IRTA, using the technology and results of the innovative pilot project, financed by the Department of Agriculture of the Generalitat de Catalunya in 2013 (File No. 56700362013). These techniques are based on quantitative PCR and allow us to determine the mycelium biomass of a fungal species, in this case Tuber melanosporum, in a soil sample (Parladé et al. 2013). The monitoring of the vigorousness of the tree will be carried out through foliar nutrient analysis.
In order to correlate the bacterial activity generated in the rhizospheric soil by the introduction of bacterial strains and the effects on the plant and the fungus, initial and final counts of viable aerial mesophiles will be carried out by means of isolating in the appropriate selective media .

Vilanova X, Morcillo M. 2013. Characterization of Pseudomonadaceae populations in Tuber melanosporum wild truffieres. 1st International Congress of Trufficulture. Teruel. Spain.
Parladé, J .; De la Varga, H .; De Miguel, A.M .; Sáez, R .; Pera, J. 2013. Quantification of extraradical mycelium of Tuber melanosporum in soils from truffle orchards in northern Spain. Mycorrhiza 23: 99-106.

Objectives

The general objective of the project is to increase black truffle production in plantations established in Catalonia through the application of selected strains of rhizobacteria and organic substances that act as triggers for the germination of truffle spores and / or potentiators of mycelial development.

The partial aims that are proposed are:

- Apply on a commercial scale, in nursery and plantation, a fertilizing product developed based on

microorganisms, truffle spores and active organic substances.
- To test the effect of the applications of different strains of rhizobacteria and organic substances separately, on the vegetative development of the black truffle (measured by mycelial quantification in the soil).
- To determine the effect of applications of different strains of rhizobacteria and organic substances separately on the improvement of mycorrhizae with black truffle in plants produced in the nursery.
- To evaluate the improvements in black truffle production in treated production plots (quantity and quality).
- To evaluate the improvement of the sanitary and nutritional status of the treated plants.
- Formulation of a commercial product in function of the previous results.

Description of project activities

In this project we will evaluate the capacity of different organic substances and rhizobacteria, some isolated from wild trunks described by Vilanova et al. (2013) in order to improve the biotic and abiotic conditions of the rhizosphere, taking into account the presence and availability of nutrients, the development of the vegetative phase of the truffle mycelium, the vigorousness of the tree (nutritional status) and the control of Pathogens.
The monitoring of the fungus response will be carried out in collaboration with IRTA, using the technology and results of the innovative pilot project financed by the Department of Agriculture of the Generalitat de Catalunya in 2013 (File No. 56700362013). These techniques are based on quantitative PCR and allow us to determine the mycelium biomass of a fungal species, in this case Tuber melanosporum, in a soil sample (Parladé et al. 2013). The monitoring of the vigorousness of the tree will be done through foliar nutrient analysis.
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The management of plant production in nursery and plantation will be carried out incorporating the biotic and abiotic components necessary to improve the productivity of the fungus and the improvement of the nutritional status of the plant. It is planned to incorporate different strains of rhizobacteria and organic substances separately and to determine their effect on mycelial biomass in the sun, the formation of ectomycorrhizas and the production of black truffle carpophores.
An overall improvement in the nutritional and mycorrhizal status of the plant is expected, which will have an impact on the higher production of black truffle carpophores. In the first phase of the project (year 1) will evaluate the effect of the different componentsseparately on the quality of the plant produced in nursery and in the field. In the second phase (year 2) will evaluate the effect of the treatments but combined over the mycelium response, moreover will control the production of truffle fruitbodies in field plantations and the nutritional status of the treated plants. In a third phase, a commercial product will be formulated and developed based on the results obtained.

Final results and practical recommendations

Experiments year 2016
The application of the 14 individual treatments on live plants during the first experiment will not cause significant differences in the concentration of mycelium of Tuber melanosporum in the growing substrate,

previously inoculate with the fungi. There are no significant differences regarding non-tractate control.
In the case of the parcels to the Granollers, a period of more than 12 years after the entry into production, cap dels tractaments aplicats will produce significant increments of the micellar concentration in relation to the non-tractate control.
In the Batea plantation, significant effects (p> 0,0008) of the different treatments on the concentration of truffle mycelium on the soil were detected.
The application of preparations of Bacillus liqueniformis (TR6), Pseudomonas fluorescens (TR9) and Saccharomyces cerevisiae (TR8) produced a significant increase in the concentration of truffle mycelium in the soil, especially in the first two treatments.
All the organic compounds that were applied (from the TR10 to the TR15) increased the concentration of truffle miceli in the soil with respect to untreated control.
In the case of the plot established in Granollers, with plants more than 12 years old, already in production, none of the treatments applied produced significant increases in the concentration of mycelium in the soil compared to the untreated control.

Experiments year 2017
During the second year of experimentation, in which combinations of rizobacteria and organic compounds were applied, significant differences between treated plants and untreated controls were detected in the concentrations of Tuber melanosporum mycelia, both in nursery plants, as in those of the plantations of Batea and Granollers.
In any case, however, the detected differences meant an increase in the concentration of mycelium in soil or substrates. The differences detected showed a decrease in the amount of mycelia compared to the controls, such as treatments TR4, TR6, TR7, TR8, TR9, TR10 in nursery, treatments TR1, TR2, TR3, TR4, TR5 and TR8 in the planting of Batea, and the treatments TR1 and TR2 in the plantation of Granollers.
In the nursery plants, treated during the second year of experimentation with different combinations of rizobacteria and organic compounds, no significant differences were detected in the quantities of total bacteria, grams and actinomycetes in the substrate of growth between plants subjected to Different treatments and control plants.
If, however, significant differences were observed in the concentration of total fungi in the plants treated with combinations TR3, TR6, TR7 and TR9. In these cases, the concentration of total fungal propanol in the substrate was superior to that of untreated control plants.
In Batea's plot, significant differences were observed in the quantities of total and gram-negative bacteria in the treated plants with respect to untreated controls.
All treatments, except TR4, increased the concentration of total bacteria in the soil, and TR2, TR3, TR6, TR7 and TR9 treatments also showed increases in gram-negative bacteria. Significant increases in concentrations of actinomycetes and fungi in the soil were not observed in any of the treatments applied.
Granollers plot revealed significant differences in the quantities of total bacteria and gram negative bacteria in the treated plants with respect to untreated controls.
All treatments, except TR9, increased the concentration of total bacteria in the soil, and all treatments, except TR8 and TR9, also showed increases in gram negative bacteria. Significant increases in concentrations of actinomycetes were not observed, however.
Contrary to what was observed in Batea, the amounts of mushroom propagules in the soil were also increased in many of the treatments applied, except for the TR9.

Effect of the applied treatments in nursery and field on the content of nutrients in the plants.
In nursery plants, more effects of applied treatments are detected than in the other two experimental situations. Significant effects on nitrogen, phosphorus, potassium, iron, boron, molybdenum or sodium were collected, but in scattered treatments without a clear relationship between them.
In the plot of Batea, the effects were minimal, and only an increase in the capture of calcium and iron was detected when applying the TR6 treatment.
The Granollers plot also detected very few effects. Some treatments, which did not coincide with each

other, showed significant increases in the content of calcium and potassium in the treated plants.

Conclusions

The application of the different rizobacteria, organic compounds, and combinations of treatments in the first and second year of experimentation did not present effects on the concentration of Tuber melanosporum mycelium in the substrate of the plants produced in nursery. The treatments used, in isolation or combined, have no effect on a young plant growing in the nursery. The conditions of the nursery (substrate, irrigation, fertilization) and the fact of incorporating a can concentration of spores of the mycorrhizal fungus to obtain the mycorrhization of the plants are sufficiently developed, and the application of this compounds in the phase of Nursery does not represent any detectable improvement.
In the plot of Granollers we find comparable results. None of the applied treatments, separately or in combination, have increased the concentration of truffle mycelium in the soil surrounding the treated plants. In a plot already established for years and that is already producing truffles, the incorporation of rizobacterial and organic compounds has no stimulating effect on the development of mycelium fungus. A plot established more than 12 years ago, the fungal composition of the rhizosphere of plants is sufficiently stabilized to be able to significantly modify the equilibrium established with contributions such as those proposed.
In the case of Batea's newly established plot in which the treated plants had an age of 3 years, the effects of the incorporation of rizobacteria such as Bacillus liqueniformis or Pseudomonas fluorescens, as well as the incorporation of organic compounds of different origins, had a stimulating effect on the development of Tuber melanosporum mycelium. In young plants, in the phase of settling in their place of definitive plantation, with strong biotic and abiotic competition of the environment, the application of these products if it confers a positive effect on the development of the mycelium of truffle .
In conclusion, the application of these products in young plantations in the phase of establishment would be recommended, and it would not be necessary in the phase of production of mycorrhizal plant in nursery or in plantations already adult in phase of production.
In view of the results obtained, it can also be concluded that combinations of rizobacteria and organic compounds did not present the expected summation effect in a principle. The hypothesis that the combination of treatments with stimulating effects on the development of truffle mycelium would have a better behavior than the components separately has not been confirmed. More studies would be needed to design, with more data, these possible combinations.
On the other hand, combinations of treatments, especially those that contained organic compounds, have had an effect on the bacterial populations of the soils of both plantations. And also the fungus in the case of the Granollers plantation, consisting of adult plants in production. It is described in the literature that these bacteria can have a positive effect on the development of the plant, and indirectly on the production of truffles. The future monitoring of these parcels would allow to verify it. At the time, to continue collecting data from these experiments, would allow it to determine if the effects of the treatment persist in time or the need to repeat them periodically during the first phases of planting establishments should be considered.
With regard to the improvement of the collection and accumulation of nutrients in plants, in all cases the detected effects were not consistent and did not allow to detect a treatment that was efficient in improving the content of nutrients in the plants. There are not enough data to extract solid conclusions.

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Tarragona	Vallès Oriental
Barcelona	Terra Alta

Project dissemination *(publications, seminars, multimedia...)*

Pàgina web del projecte

Other project information

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