6.11

Analysis to control and monitor a truffle plantation

There are basically two types of routine analysis that can serve as a guide in this phase:

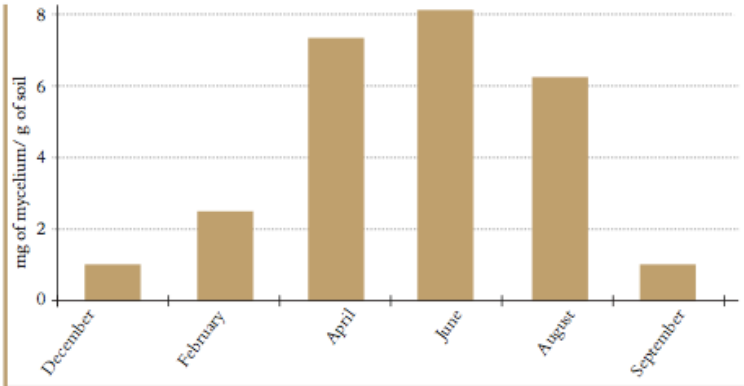
a) *Mycorrhization status analysis*. This indicates the level of truffle colonisation in the host tree and its potential to produce, as well as the levels and relative proportions of other contaminating fungi. However, some studies show that truffles fruit in areas with a low density of fine roots and near to long unbranched roots that are not mycorrhized but have stroma.

b) *Mycelium quantification using molecular analyses*. Molecular analyses are based on quantitative PCR. This technique is harmless to the plantation, and it is simple, as the truffle farmers can gather the necessary soil samples themselves. Once in the laboratory, the truffle is amplified for species identification and compared against a standard curve with known amounts of mycelium. The authors of this book, together with researchers from the Institute for Food and Agricultural Research and Technology (IRTA) in Barcelona, are creating the standard reference curve for *Q. ilex* in *truffières* of different ages, by comparing plants with and without production. However, these curves will need to be improved with more data from host trees of other species and from different climates and countries.

Other data, like those produced by Ion-Torrent technology, allow the diversity of fungi in a plantation to be studied, and detection both of other contaminating fungi and beneficial bacteria. As these tools become less expensive they can be used to fine tune plantation management. For example, the distance at which the mycelium is growing from the tree trunk, and in what amounts, can be evaluated in order to make changes to the irrigation system or the distance and depth of tillage.

A study using these techniques observed 3 year-old holm oaks to have 1,000 times more mycelium at 40 centimetres from the trunk than at 1 metre, while at 5 years of age this difference was down to 100 times. At 7 years of age, there was no longer any difference in the amount of black truffle mycelium at 40 cm, 1 and 2 metres from the trunk.[133] The truffle mycelium appeared to stabilise at a certain distance. The authors conclude that there may be a certain load capacity after which no more mycelium is produced.

An Italian study found a greater quantity of black truffle mycelium in productive burns, but it is not known



Development over the year of black truffle mycelium in the soil of a 4 year-old holm oak plantation with drip irrigation.

whether this quantity represents the threshold mentioned above[134]. Our research in a plantation of 12 year-old holm oaks without irrigation shows the average amount of mycelium in summer for non-productive trees to be 14 mg per gramme of soil, while for productive trees it is 24 mg per gramme of soil.

Initial studies show that wild *truffières* have up to ten times more mycelium than cultivated grounds,[93] which appears logical considering that these truffle strains have to adapt to the area and so have to be more vigorous, while in the plantation man has 'helped' the fungus to develop. This same study finds more truffle mycelium in irrigated than in unirrigated plantations.

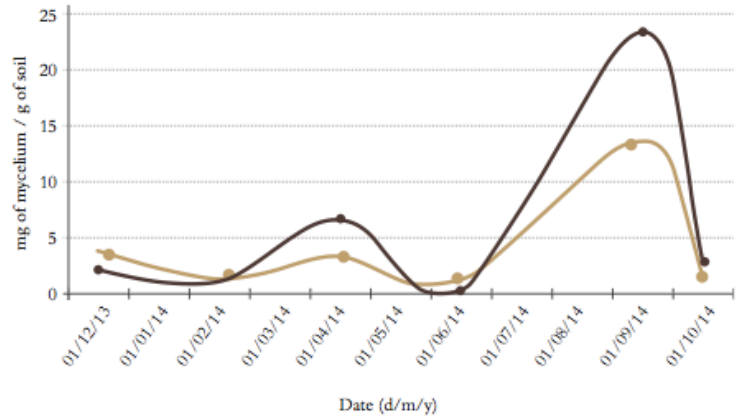
Other studies find a clear relationship between the amount of extra-radical mycelium and the formation of truffles, both in *T. melanosporum*,[135] and in *T. magnatum*.[136]

An unpublished study by the authors of this guide analysed 32 truffle grounds in Spain. The results for the 159 trees analysed were *Tuber melanosporum* was present in 83.6% of the

Although the black truffle is maintained at good levels in all types of plantations, more and more *Tuber aestivum* are being found in unfenced plantations where wild boars can enter freely. This is one more reason to recommend fencing at the outset.

trees, meaning that 16.7% of these trees had no truffles. *Tuber brumale* was detected in 12.6% of the trees and *Tuber aestivumin* 10.1% of them. 25.2% of the trees had other mycorrhizal fungi and only 31.4% of the trees had no contaminants of any type.

A similar study in Australia[130] found that less than 70% of the nursery seedlings and trees in the field had *T. melanosporum* and that some trees had been contaminated by *T. brumale*, presumably during the inoculation process.



Comparison of the evolution of the mycelium concentration of *T. melanosporum* in the soil of producing and non-producing 11 year-old holm oaks in a plantation without irrigation.
—Producers —Non-Producers