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Empirical or rational truffle cultivation? It is time to choose

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Abstract

Aim of study: The aim of this study was to finalize a new method of truffle cultivation in order to obtain an earlier, more regular and sizeable production of high quality fruiting bodies.

Area of study: The experimentation was carried out in France (country of Dordogne, south-western France) and Italy (country of Marches, eastern central Italy) for more than one decade.

Material and methods: For the first time the method is based on scientific data on truffle biology particularly: the dynamic system of mycorrhization by the truffle and by other fungi; the saprophytic capability of the truffle; the ability of its mycelium for decomposing certain minerals and organic materials in the "brûlés".

The basic principle concern the work of the soil and the upkeep of the root system: to work the soil immediately after the plantation of mycorrhizal seedlings, deeply enough, with adapted tools, in order to do not compact the soil, aerate it, favour the production of deep fruiting bodies not exposed to high temperatures, dryness, frost, parasites…; cut accurately the roots in order to regenerate them and consequently to provide food for the truffle mycelium.

Main results: The result has been a new cultivation method designated "differentiated" and called "MRT", with adapted work of the soil on the lines of plantation and upkeep of the grass between them, to maintain the cohabitation between areas where the mycelium is present, from those where it is absent.

Research highlights: Research is going on to improve the techniques and particularly finalize tools more precise and more adapted for working the soil and maintaining the root system in a best way.

Key words: truffle; production; nutrition; soil; root system; cultivation tools.

Introduction

The most important advance in technology in truffle cultivation has been the large scale introduction of the inoculated seedling, of which we have celebrated its 40th birthday last year. Over 90% of truffles harvested in France come from plantations, wild truffle areas becoming rarer and rarer.

If the use of mycorrhized seedlings has given excellent results, failures have been just as frequent. The reasons are now known.

From the beginning of the introduction of the mycorrhized seedling, J. Grente (1974), from INRA Clermont-Ferrand, drew-up a guide for the optimal use of this new tool, based on three principles: the clever choice and eventually the adaptation of the environment (climate, topography, altitude, soil), the choice of the truffle species best adapted to that environment and finally the maintenance of the conditions to favour mycorrhization, then the fruiting bodies. Those principles are still current.

If numerous failures have been due to a bad choice of environment (in particular acid soils) or the bad quality of mycorrhized seedlings, the majority of failures have been attributed to the absence of adaptation in cultivation practices.

Current methods of truffle cultivation

In the second half of the last century, truffle cultivation is still using methods inherited from the Ancients. "The ancestors'cult has prevailed for a long time (and still prevails some of the time) in the French truffle cultivation, leading to old fashioned practices inspired from the beginning of the XXth century" (Olivier *et al*., 2012). The reference manuals are in

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Figure 1. Truffle cultivation according to the «Pallier method» (country of Teruel, Spain).

Figure 2. Trufffle cultivation according to the «Tanguy method» (Quercy, South West of France).

France those of Rebière (1967, 1981) and in Italy, those of Mannozzi-Torini (1970, 1988). The common point to all methods in these manuals is the working of the "superficial soil, so that you avoid damaging the rooting systems" of the truffle trees, with agricultural tools used for industrial agriculture.

With the event of the mycorrhized seedlings in 1973, the end of the 1980's has seen new methods appearing (Sourzat, 2002, 2012). The "Pallier system" is a method of transfer that mimics and rationalises the methods from arboriculture to truffle cultivation: mycorrhized seedlings planted in calcareous favourable soil, soil worked diligently and adapted, controlled irrigation, pruning frequent and severe to keep the environment 'open', fertilisation and soil additions, phytosanitary treatments (Fig. 1). In effect, this method copies those proposed by Rebière and Mannozzi-Torini, with modern tools of large scale plantations, with the novelty being the use of the mycorrhized seedlings. The economics of the Pallier system have been satisfactory. The advantage of this method is a relatively early harvest (5-6 years).

At the end of the 90's other concepts of truffle cultivation appeared, better "ecologically", truffle cultivation on calcic grassland or "system Tanguy". In this system, mycorrhized seedlings are planted, with the soil cultivation stopped when the trees have taken. The area is then left to grow (artificially or seeded) mainly with grasses (Fig. 2). The production starts rather late and remarkably high per tree.

The "system Malaurie" develops poor calcareous areas with truffle plantations that allow the best technical-economic productivity. The characteristics are mycorrhized seedlings, sparing water by excluding irrigation, cutting the grass or working the soil, with a rationale based on incurring the least expense for maximum results and the conservation of a typical landscape for agro-tourism.

The systems used in Italy, Spain, Australia, New-Zealand, Sweden, U.S.A. are copies of the Pallier system (Bencivenga *et al*., 2012; Granetti *et al*., 2005; Hall *et al*., 2007; Reyna *et al*., 2007; Weden, 2008). All the systems result in good rate of production, however they are very inferior to those before the First World War in France.

The defects of current methods

The main reproach to make to the different methods is that they work blindly, leaving aside the truffle biological requirements. In 1981, Rebière writes: "Due to the lack of precise data on the truffle biological cycle, and mostly on the mycelium evolution in the soil, waiting for the identification of the best periods, we will refer ourselves to the methods used by our old truffle growers for the maintenance of the truffle plantations". "From the technical point of view, the ancestor's cult has prevailed a long time (and still does sometimes) in the French truffle cultivation, leading to practices sometimes old-fashioned inspired from the beginning of the XXth century" (Olivier *et al*., 2012). This is no longer the case, and truffle research has progressed substantially.

The "system Pallier" implies the work of the whole plantation, this means areas where the truffle mycelium

has not yet reached, but where numerous "dormant" contaminant mycorrhizal fungi (*Melanogaster, Hymenogaster*...) or true truffles but of a lesser quality to *T. melanosporum* (*T. brumale, T. aestivum)* could be. The disorganised cultivation uproots the truffle ecosystem and the propagules of the various fungi present in the plantation are spread by the agricultural equipment. The plantation ends up infected by the contaminants; this has often been noted with *T. brumale* (Sourzat, 2008). Another disadvantage is the high cost during ground works.

The main defect of the methods based on intensive agriculture in truffle plantations is the excessive mechanisation, with equipment adapted to large scale agriculture, but not those of the truffle, which leads to disastrous consequences on the life of the truffle plantation: compact soil and root system destroyed.

The "system Tanguy" is the opposite. Since there is no ground works, the colonisation of *T. brumale* does not exist (Sourzat, 2008). The reduction in frequency of the works means that it is less expensive. The main disadvantage is the long delay between the plantation and the first harvest (over 10 years, sometimes 20 years!) and the growth of truffles close to the surface. This means low truffle quality due to exposure to heat, dry weather, frost and predators. This method of abandon of the truffle orchard after plantation (except of the control of the grass by cutting), is it a true truffle cultivation method, or rather a practice of maintained fallow?

The scientific basis for a new method

Amongst the scientific factors on which the new method rests, three can be considered as determinant factors: the evolution of the biological behaviour of the truffles as they develop, the truffle mycorrhization dynamic and its competitors, and the truffle mycelium's activity in the brûlé.

The saprophytic behaviour of the truffle

The truffle mycelium behaves symbiotically, but changes quickly towards a saprophytic behaviour. It then feeds from elements of the rooting system, in particular the tannin. It consumes the nourishing elements in the dying roots: complex carbohydrates, nitrogen and minerals trapped by tannins (Pargney *et al*., 2001a, 2001b). The carbon nutrition of the truffles comes from the tree (Le Tacon *et al*., 2013) but the truffle shows a saprophytic behaviour for the organic debris in the soil: old roots and complex organic materials during degradation (Barry *et al*., 1993; Bouzama, 2004; Pargney *et al*., 2010)

The "wearing off" of the root system starts at the trunk, and spreads in the manner of a centrifuge, following the progression of the mycelium front, when there are no more roots to devour, the truffle disappears (Dessolas *et al*., 2007; Pargney *et al*., 2010). This phenomenon explains why isolated trees, of which the centrifugal progression is not disturbed, can produce truffles continuously. On the contrary, in plantation, when the brûlés join-up, the production goes down markedly, then stops. The truffle has nothing to "eat". It is therefore imperative to find a method of cultivating, to master the root system of the tree, itself linked to the aerial system of the tree, on one hand and on the other hand regenerate the rooting system.

The mycorrhization dynamics by the truffle

It is dependent on the root system development of the host plant, itself linked to its aerial system. When a tree grows too fast, the mycorrhization by the truffle does not 'follow' any longer, does not keep pace, and the extremities of the roots catch foreign mycorrhizal fungi, perhaps less interesting truffles. The truffle mycorrhization lags always behind, in relation to the root front. It is necessary to manage the rate of growth of the root so that there are no terminal zones lacking truffle mycorrhiza (Chevalier, 2008).

The brûlé

In creating a brûlé, the truffle "prepares its nest". The mechanisms are not all known. It is certain that the truffle mycelium has a phytotoxic effect on the vegetation, but the mycelium also provokes physical and chemical effects on the soil. The environmental conditions created by the brûlé are very advantageous to the vegetative development of the truffle and to its fruiting. The mycelium, in the brûlé, causes a modification of the structure of the soil surface, making it lighter. It attacks the organic matter: the percentage of organic matter in the brûlés is lower than outside of them, in addition (Callot *et al*., 1999; Ricard , 2003),

Figure 3. Differential cultivation with a mulch of gravels on the line of plantation (Perigord, South West of France).

Figure 4. Differential cultivation with a mechanical work on the line of plantation (Provence, South East of France).

in the grassy area its composition is different. The mycelium degrades minerals (clay, micas...) (Neel *et al*., 2007). Finally, the truffle development leads to the formation of important quantities of active calcium and exchangeable calcium that gives it an advantage over other mycorrhizal fungi and to their detriment by a feed-back effect (Garcia-Montero *et al*., 2007).

A new method, the "rationale method of truffle cultivation" (MRT)

The MRT ("méthode rationnelle de trufficulture") breaks away energetically from the empirical methods still largely practised today. Overall, Gregori's "integrated method" proceeds on the same principles (Gregori, 2008) .

A fundamental point is the necessity of "differential" ground works in truffle plantation, by treating differently the zones where the truffle mycelium is present and those where it is absent (Chevalier, 2008; Chevalier 2009; Dessolas *et al*., 2007; Pargney *et al*., 2010). In effect, it is a compromise between the system Pallier where it is "all ground works", and the system Tanguy with "all grassland". Ground works is practised along the plantation lines, and grass is maintained in-between the lines (Figs. 3 and 4).

A second essential point is a break-off from the universal dogma of working the soil "superficially", which excludes the brûlés, so that the rooting system is not damaged. On the contrary, it is necessary from planting in the first year, to work the soil around the trees, sufficiently deep. The advantages of working the soil are numerous: competitive grasses eliminated, soil aeration, increased penetration of rain water, limited evaporation, production of truffles deeper and of good quality, instead of surface truffles, degraded by heat, drought, frost and attacked by parasites (small mammals, flies, liodes, slugs...). If they are not harvested and they are immature, they rot before being ripe. Another advantage of ground works is derived from the regeneration of the root system when the plantation ages, to form new functioning mycorrhiza, with adapted tools, so that production does not stop. Finally, working the soil contributes to "help" the truffle to make its brûlé, which requires a great deal of energy, in heavy clay soil without stones. The principle of the Ancestors"to laissez-faire nature" risks, in this type of soil, to lead to very late production or none.

The third point which diverges with traditional methods relates to the tree pruning (Fig. 5). This must be energetic and can be done in any season. The aims are multiple: to limit the tree development and foliage and indirectly the root system, to lead to the formation of a lateral root system by polling the tree (Rimbault, 2003; Drenou, 2006), indirectly thus stimulating and encouraging the formation of rootlets, to aerate the centre of the tree to allow sunrays and rain to go through, to limit the water evaporation in the summer, to provide a mulch with cut branches to protect the brûlés in summer. Different methods of "bonsai" cuts are being used or tested in experiments. The rehabilitation of the ground works leads to the problem of which equipment to use. If the manual work around the trees is preferable, this is not possible over large areas. The equipment used in truffle cultivation, be it tine or discs are

Figure 5. «Bonzai» pruning of the tress (Rocca Fluvione, central eastern edge of Italy).

Figure 6. Becker equipment.

adapted to industrial agriculture, and not for truffle cultivation. Moreover they are pulled by a tractor, and tractors compact the ground, and furthermore, compaction is the worst enemy of the truffle.

For a few years, agricultural equipment that does not compact the ground are being tested. They are

Figure 7. Dessolas equipement.

carried by diggers on tracks (equipment Becker, Fig. 6), either pulled behind or aside (equipment Dessolas, Fig. 7; equipment Chabert, Fig. 4). The use of these equipments is still too recent to choose the best performing one, however, there is a tendency to select the disc type which cuts swiftly and cleanly the root system and allows its regeneration, contrary to equipment with tines which damage it.

Results

The improvements cover different areas: truffle quantity harvested, quality, length of time for truffle plantation viability, "ecological" character.

For the quantity, the first truffle harvest is much more early than in traditional methods (4 years after planting, or even 3), whatever the species of the host tree used. The pubescent oak is just as early as the hazel, the hornbeam, or the evergreen oak. The number of productive trees is higher (70-80% four years after planting).

Considering quality, the ground works in depth leads to deep truffles, not subject to high temperatures, lack of rain, cold and predators. The mechanical regeneration of the root systems and the management of tree density to avoid the "closure" of the canopy in truffle plantations, are the guaranties for a truffle cultivation of durability, capable of continuous production for decades, whilst in a truffle plantation with no maintenance, production stops after twenty years or so.

Finally, a clever mechanical work eliminates the use of treatments. It allows the reduction of weed and grass killers, even in totality, deep soil working allows also a substantial saving in water resources. In 2003, year of severe drought, some pioneer truffle growers have

been able to obtain a good truffle harvest without watering.

Conclusion

Rational truffle cultivation implies leaving aside links with empirical methods used up to now, which give unreliable results, and to take into account the most recent scientific data on truffle biology. Between the system Pallier meaning "constant work", and the Tanguy system, "all grass", the solution is differentiating the production zones and those that are not yet, or not any longer producing. The management of the ground works needs to be carried out in relation to the dynamic development of truffle mycorrhization on the root system. The dogma of superficial work, so as not to damage the root system, must be dropped. On the contrary, it is necessary to work the soil sufficiently in depth with adapted tools. It is also a necessity to re-generate the root system when the truffle has "worn it out".

The observation of three essential points, differentiated management of the truffle plantation, ground works sufficiently deep, use of adapted equipment, is the key to intensive truffle cultivation. The Europeans who do not have vast areas to grow truffles, like certain countries of the South hemisphere, will stay competitive only if they use effective methods, allowing an early harvest, of quality and long life.

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