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Trapping truffle production in holes: a promising technique for improving production and unravelling truffle life cycle

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Abstract

The Périgord black truffle, *Tuber melanosporum* Vittad., is an ectomycorrhizal fungus that forms edible hypogeous ascomata. It is now harvested in plantations and is recognized as an agricultural product by European policy. Empirical techniques without scientific demonstration of their efficiency are often used to improve the production of truffles in plantations. One of these techniques is "truffle trapping" which consists in practicing holes inside the potential productive area and to fill them with a substrate containing ascospores. We report an experiment in a truffle orchard where 784 holes were set under 196 trees. Two years after the installation of the holes, 95 % of the truffles were found inside the holes corresponding to only 5 % of the productive area. This study confirms the efficiency of this empirical technique and demonstrates new ways for *in situ* studies of the truffle life cycle.

Keywords: truffles; orchards; cultivation technique; trapping production

Riassunto

Il tartufo nero pregiato, *Tuber melanosporum* Vittad., è un fungo ectomicorrizico che forma corpi fruttiferi eduli. Attualmente viene raccolto in apposite piantagioni ed è riconosciuto come prodotto agricolo dalle politiche europee. Per incrementare la produzione di tartufi in queste piantagioni vengono spesso utilizzate tecniche ancestrali la cui efficacia non è mai stata scientificamente provata. Una di queste tecniche si basa sulla realizzazione delle cosiddette "trappole da tartufo" e consiste nella realizzazione di pozzetti nei quali viene aggiunto un substrato contenente ascospore. In questo articolo viene illustrato un esperimento realizzato in una piantagione nella quale sono stati realizzati 784 pozzetti in corrispondenza di 196 alberi. Due anni dopo la realizzazione delle trappole, il 95% dei tartufi sono stati raccolti all'interno dei pozzetti, che nell'insieme rappresentavano soltanto il 5% dell'area potenzialmente produttiva. Questo studio conferma l'efficacia di una tecnica ancestrale ed illustra nuove metodiche per lo studio *in situ* del ciclo biologico del tartufo.

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Parole chiave: tartufi; piantagione; coltivazione; produzione tramite trappole

Introduction

Truffles are fungi that belong to the *Tuber* genus and form ectomycorrhizal associations with numerous trees or shrubs (Mello et al., 2006). Some species are famous throughout the world for the organoleptic properties of their ascomata such as the black Périgord truffle, *Tuber melanosporum* Vittad., which is naturally harvested in Europe under Mediterranean climate. Since 2015, the truffle has been recognized as an agricultural product, and truffle orchards realized by inoculated seedlings are considered as a perennial culture that allow farmers to obtain European Union financial support in the common agricultural policy. Since 1970s, it is possible to inoculate seedlings with *T. melanosporum* in well-controlled conditions (Chevalier and Grente, 1979; Murat, 2015). The inoculated seedlings are then transplanted in the field. Due to the plantation effort, truffle orchards also turn the agricultural landscape in sustainable agro-forestry ecosystems, maintaining local activities and landscape management in fire-prone regions. Now, in France, 80 to 90 % of the *T. melanosporum* ascomata are harvested in such truffle orchards (Olivier et al., 2012). Using seedlings previously inoculated with *T. melanosporum* in nursery, this species was introduced in many countries all around the world, including Australia, USA, South Africa, and New Zealand. *Tuber melanosporum* is agro-food representing opportunities of incomes and agricultural diversification for rural populations. However, clear guidelines for *T. melanosporum* orchards management are lacking and, with the exception of irrigation (Le Tacon et al., 1982) and soil tillage for *T. magnatum* (Salerni et al., 2014), the management (e.g., soil tilling and tree pruning) relies on empirical techniques. In the eighteenth century, Buffon (1749) transplanted one *boisseau*, old French measure corresponding to 12.66 litres, of truffles with their surrounding soil in a garden under hornbeam. Two years later, he harvested four *boisseaux* of truffles. This experiment was the first to describe "truffle traps", consisting in holes filled with a mix of substrate (i.e. peat, vermiculite…) or soil and spores. Now each truffle grower develops his specific protocol of truffle trapping, but this technique has never been experimentally studied at a large scale.
The aim of this study was to test the efficiency of this empirical agricultural technique and to determine whether truffle ascomata production in a hole (i.e. in a restricted area) is possible. The possibility to trap truffle production in few cm^2 could open new perspectives for truffle life cycle unravelling.

Materials and methods

This study was carried out in March 2013 in a 3.5-ha truffle orchard planted in 2007 with 1 and 2 years-old *Quercus pubescens* seedlings, previously inoculated with *T. melanosporum* spores. The plantation is composed of 945 trees distributed in 7 rows of 78 trees, 2 rows of 79 trees, 2 rows of 80 trees, and one row of 81 trees. The experimental site is located in West Atlantic France, but its exact position cannot be given, since the orchard owner wanted to remain anonymous. One hundred ninety-six trees out of 945 were selected (the first two rows and half of the plantation) for the study. The substrate used to fill truffle holes was prepared in a cement mixer with 250 g of crushed *T. melanosporum* ascomata kept at -20°C several months until their use, 350 g of honey, 50 l of horticultural vermiculite, and 50 l of organic compost. This substrate was introduced in 4 holes for each selected tree at North, South, East and West at 50 cm from the tree trunk. A clod of ground of 0.20 m of sides and 5-8 cm of depth was removed using a shovel, which was used to cut the roots and the mycorrhizas. Two hundred fifty ml of substrate was deposited at the bottom of the hole and

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the clod was handed over. The surface of the holes per tree was 0.16 m^2, representing 5% of the average surface of the "brulés" (i.e., area around the tree without vegetation typical of black truffle corresponding to the theoretical productive area). Truffles were harvested in the entire plantation during 2012-2013, 2013-2014, 2014-2015, and 2015-2016 seasons (Tab. 1) with a truffle-hunting dog. The experimental design did not allow to robustly comparing the truffle production of the area with holes versus the non-hole area of the orchard but data for both are presented. The statistical analyses were conducted using R (Venables and Smith, 2005) and the Wilcoxon test or chi square test of the STATS package (R core team, 2014).

Results

The production of truffles began in 2012-2013, five years after the plantation (Tab. 1). The holes were installed in March 2013 and the year after (season 2013-2014); the truffle production of the orchard was weak (2 kg; Tab. 1). In the second year following the installation of the holes, the truffle production of the orchard had deeply increased (26.7 kg). The 196 trees with holes produced 11.8 kg of truffles (16.7 kg/ha) of which 11.3 kg (95.6%) were harvested inside the holes compared to 0.51 kg (4.4%) outside. Expressed in grams of truffle per square meter, these differences were highly significant (362 g/m^2 in the holes versus 0.9 g/m^2 outside the holes; p-value = $2.2e^{-16}$; Fig. 1). In some holes, more than 10 truffles were harvested as illustrated by the movie 1 and it was commonly found more than 4 truffles in one hole (Fig. 2; video in supplementary file). In the third year following the installation of the holes, the truffle production of the entire orchard had decreased in parallel with a decrease of the proportion of productive trees (probably linked to weather factors as everywhere in France). The production remained mainly localized in the holes (35 g/m^2 in the holes versus 4 g/m^2 outside the holes; p-value = 0.01; Fig. 1).

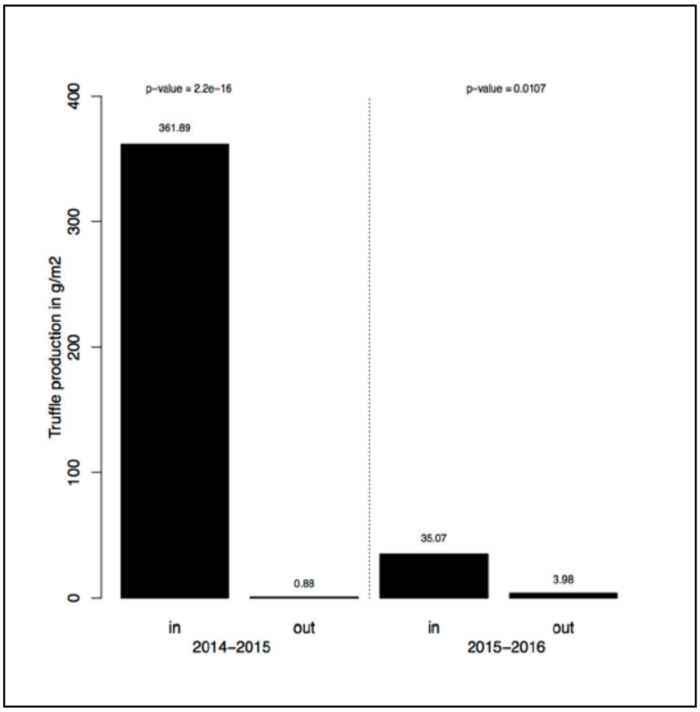

Fig. 1. Truffle production in g/m^2 inside the holes (in) and outside (out) under the productive trees of the area with holes. The p-values of the chi square test are indicated for both seasons.
Fig. 1. Produzione di tartufi in g/m^2 all'interno (in) ed all'esterno (out) dei pozzetti in corrispondenza degli alberi produttivi nell'area nella quale sono state realizzate le trappole. L'analisi statistica (test del chi quadro) è riportata per entrambe le stagioni.

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Although we are aware that the experimental design (e.g. absence of repetition blocks) does not allow comparing robustly the production between holes and non-hole trees, the production data and comparison between both areas are showed in Tab. 1. Our data suggested the production was higher in hole area due to the increase of the number of productive trees (27.6 % in the hole area against 9.8 % in the area without holes in 2014-2015, p-value = 0.001 and, 7.7% against 2.8% in 2015-2016, p-value = 0.006; Tab. 1).

Tab. 1. Evolution of the truffle production in the whole orchard, including in the area with holes and in the non-hole area: total production per season in kg and in kg/ha (brackets), percentage of productive trees, and mean truffle production per productive tree in grams.
Tab. 1. Evoluzione della produzione di tartufi nell'intera piantagione (area con le trappole ed area priva di trappole): produzione totale in kg ed in kg/ha (parentesi) per ciascuna stagione, percentuale degli alberi produttivi e produzione media (in grammi) di tartufi per albero produttivo.

		Hole area	**Non-hole area**	**Whole orchard**	**p-value** * chi square ** Wilcoxon test
Total number of trees		196	758	954	
Total truffle production in kg and (kg/ha)	2012-2013	0.006	0.024	0.03	
	2013-2014	0.41	1.59	2	
	2014-2015	11.86 (16.7	14.81 (5.38)	26.67 (7.62)	$2.2e^{-16}$*
	2015-2016	3.74 (5.27)	5.60 (2.04)	9.34 (2.67)	$2.2e^{-16}$*
Percentage of productive trees	2014-2015	27.6	9.8	13.4	0.001*
	2015-2016	7.7	2.8	5.3	0.006*
Mean truffle production per productive tree in g	2014-2015	208.3	200.1	208	0.974**
	2015-2016	124.8	266.6	183	0.013**

Fig. 2. Pictures showing the truffles harvested in holes
Fig. 2. Immagini che mostrano la raccolta dei tartufi nei pozzetti

Discussion

Trapping truffle production in holes is a common practice realized by truffle growers but until now its efficiency was not demonstrated. In this preliminary experiment we demonstrated that on 196 trees in one orchard, 95.6 % of the production occured two years later inside the holes (representing only 5 % of the productive area of the trees). The installation of truffle holes leads to the modification of several parameters: soil structure, soil fertility, content of organic matter, pH, etc. Consequently, all these factors could explain the truffle production inside the holes. Our analysis aims to demonstrate the effectiveness of trapping truffle production in holes and not to discriminate among all these factors that's why controlled holes (e.g. without spores or without honey) were not realized. Nevertheless, the role of ascospores in the initiation of the sexual reproduction introduced in the holes seems particularly important to discuss. Indeed, recent analyses suggested that *T. melanosporum* ascospores together with conidia or soil mycelium could act as male elements (Taschen, 2015; Le Tacon et al., 2015; De la Varga et al., 2016). Although hermaphrodite strains exist, they are not frequent and a specialisation for male or female strains is observed (De la Varga et al., 2016). The important genetic diversity and turnover of genotypes suggest that germinating *T. melanosporum* ascospores probably act as male elements. Considering the current knowledge of *T. melanosporum* life cycle it is tempted to propose that the great number of truffles found in these holes could be explained by the presence of numerous ascospores. Indeed, many of them could be still able to germinate and to act as male element to fertilize the female structures produced by the mycorrhizas. In the third year after the installation of the holes, there was still a positive effect of the treatment, but to a lesser extent, probably because of an insufficient number of surviving ascospores able to play the role of male elements.
The possibility of trapping truffle production in a few cm^2 presents a new method to understand the life cycle of the truffle. For example, the role of ascospores in sexual reproduction as female and/or male partners could be investigated be setting up holes with previously genotyped ascospores, allowing to determine their contribution to the formation of maternal structures (i.e. ectomycorrhizas and gleba of ascomata) and/or male elements. The understanding of the *T. melanosporum* sexual reproduction will lead to optimize the cultivation of this species in orchards.

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References

BONNEAU, L. 2016. A propos des apports de spores dans les brulés. L'historique et le vécu "en temps reel" par l'expérimentateur. Le Trufficulteur 94, 9-12.

BUFFON, G., SONNINI, C.S., LATREILLE, P., 1749. Histoire Naturelle Générale et Particulière Avec La Description Du Cabinet Du Roi (1749 à 1789). Histoire Naturelle Des Plantes. Tome Troisième. Plantes Cryptogames, Des Champignons. p. 37.

CHEVALIER, G., GRENTE, J., 1979. Applications pratiques de la symbiose ectomycorhizienne: production à grande échelle de plants mycorhizés par la truffe (*Tuber melanosporum*). Mushroom Science 10, 483–505.

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De la Varga, H., Le Tacon, F., Lagoguet, M., Todesco, F., Varga, T., Miquel, I., Barry-Etienne, D., Robin, D., Halkett, F., Martin, F., Murat, C. 2016. Investigation of the sexual reproduction strategy of the Perigord black truffle (*Tuber melanosporum* Vittad.) revealed trioecy. bioRxiv, doi: http://dx.doi.org/10.1101/073650.

Le Tacon, F., Delmas, J., Gleyze, R., Bouchard, D., 1982. Effect of soil water regime and fertilization on fructification of the black truffle of Perigord (*Tuber melanosporum* Vitt.) in south east of France. Acta Oecologica, Oecologia Applicata 3, 291–306.

Le Tacon, F., Rubini, A., Murat, C., Riccioni, C., Robin, C., Belfiori, B., Zeller, B., De la Varga, H., Akroume, E., Deveau, A., Martin, F., Paolocci, F. 2016. Certainties and uncertainties about the life cycle of the Périgord black truffle (*Tuber melanosporum* Vittad.). Annals of Forest Science 73, 105-117

Mello, A., Murat, C., Bonfante, P., 2006. Truffles: much more than a prized and local fungal delicacy. FEMS Microbiology Letters 260, 1–8.

Murat, C. 2015. Forty years of inoculating seedlings with truffle fungi: past and future perspectives. Mycorrhiza 25, 77–81.

Olivier, J.M., Savignac, J.-C., Sourzat, P., 2012. Truffe et trufficulture. Editions Fanlac, Périgueux.

Rubini, A., Belfiori, B., Riccioni, C., Arcioni, S., Martin, F., Paolocci, F., 2011. *Tuber melanosporum*: mating type distribution in a natural plantation and dynamics of strains of different mating types on the roots of nursery-inoculated host plants. New Phytologist 189, 723–735.

Salerni, E., Iotti, M., Leonardi, P., Gardin, L., D'Aguanno, M., Perini, C., Pacioni, P, Zambonelli, A. 2014. Effects of soil tillage on Tuber magnatum development in natural truffières. Mycorrhiza. 24, 79-87.

Team, R.C., 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013. ISBN 3-900051-07-0.

Venables, W.N., Smith, D.M., 2005. An Introduction to R. Notes on R. Edition Network Theory Ltd., Bristol, UK

Movie in supplementary file