Ten years of quality control of seedlings inoculated with *Tuber melanosporum* in Viveros Alto Palancia

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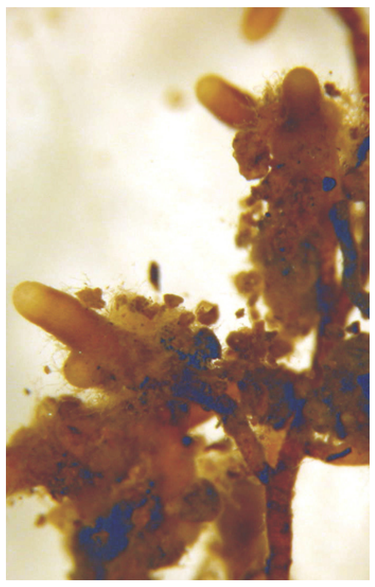
INTRODUCTION AND AIM

The successful cultivation of the black truffle (*Tuber melanosporum*) is based on the plantation of mycorrhized seedlings on suitable soils. The attributes of the nursery seedlings, particularly their mycorrhizal state, influence the performance of the fungus and the plant in the field. However, **in Spain there is not an official regulation for mycorrhized seedlings yet**. And the assessment of mycorrhization requires specialised training and equipment.

Reyna *et al.* (2000) proposed a methodology for the **quality control and assessment of truffle-inoculated seedlings**, which involved: (a) checking all the sporocarps used as inoculum, (b) assessing the mycorrhizal state of the commercial stocklots on the basis of **the number and the percentage of root tips colonised by the fungus** and the occurrence of other ectomycorrhizal fungi, and (c) assessing the occurrence of contaminating ectomycorrhizal fungi in the nursery through bait seedlings.

In 2003, the CEAM signed a cooperation agreement with the nursery Viveros AltoPalancia to apply this protocol. From then, the stocklots of mycorrhized seedlings have been checked every year. **This works presents the evolution of the mycorrhizal state of the stocklots of one-year-old seedlings of *Quercus ilex* inoculated with *T. melanosporum* from 2003 to 2012**.



The quality control of truffle-inoculated seedlings involves checking both the truffles used as inoculum and the mycorrhizal state of the commercial seedlings. A quality plant must show a high no. of mycorrhizas of the target truffle, and these mycorrhizas must occupy a high percentage of the fine roots. The occurrence of other ectomycorrhizal species must be low. No other species of *Tuber* must be present.

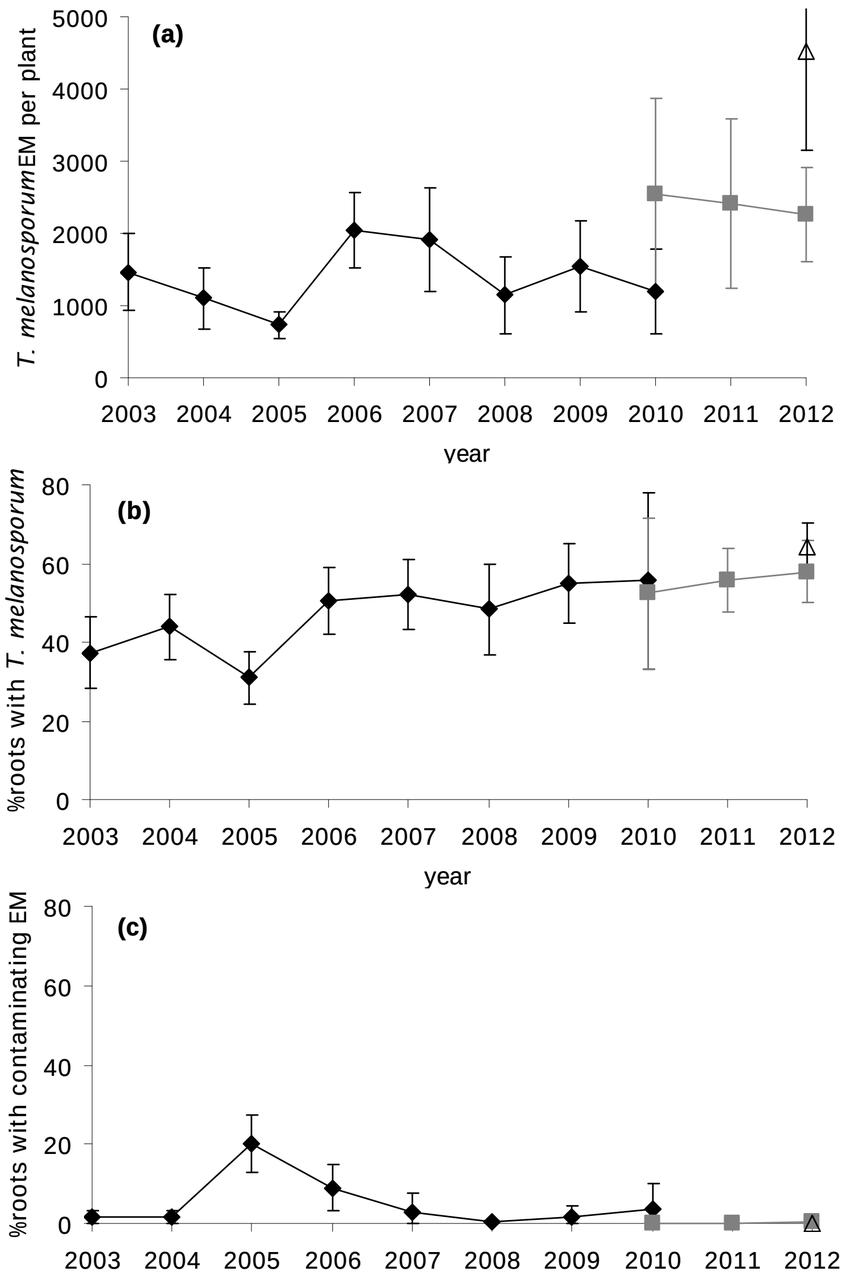


Fig. 1 Evolution of the stocklots of one-year-old seedlings of *Q. ilex* x *T. melanosporum*: the no. of *T. melanosporum* mycorrhizas (a); percentage of roots colonised by *T. melanosporum* (b); and percentage of roots colonised by contaminating ectomycorrhizal fungi. Each stocklot was evaluated by sampling 12 seedlings. **In 2010 fundamental changes in the production process were made (grey line and dots). In 2012 a change in the atmospheric environment of greenhouses was tested (empty triangle).**

EVOLUTION OF THE INOCULATED-SEEDLING QUALITY

In the ten years of existence of the nursery, two different periods are distinguished:

(1) From 2003 to 2009 the production processes and growing conditions remained substantially uniform, although some year-to-year variation was unavoidable because the plastic greenhouses lack automated environmental control systems:

–The no. of *T. melanosporum* mycorrhizas per plant remained stable around 1400 (**Fig. 1a**).
–The percentage of fine roots colonised by *T. melanosporum* slightly increased over time (at a mean rate of 3% per year) (**Fig. 1b**).
–A **sudden increase of *Sphaerosporella brunnea*** happened during the third year (**Fig. 1c**).

In this period the cultivation method was simply fine-tuned to keep down the occurrence of the contaminating fungus, by avoiding periods of constantly high water content in the growing substrate.

(2) From 2010, the focus moved to testing new production processes and improving seedling quality:

-In 2010 fundamental **changes in the production processes** were tested (grey dots in Fig. 1). In view of the positive results, these changes were implemented in 2011. As a result, the **no. of *T. melanosporum* mycorrhizas per plant increased** to ca. 2400 (**Fig. 1a**). The percentage of roots colonised by *T. melanosporum* remained at similar levels to previous years. The occurrence of contaminants (mainly *S. brunnea*) remained very low.

-In 2012 a **change in the atmospheric environment** of the greenhouses was tested (empty triangles in Fig. 1). A relevant **increase in the no. and percentage of *T. melanosporum* mycorrhizas** resulted (**Fig. 1a, 1b**). In 2013 it will be tested if this gain was independent of the annual weather conditions.

CONCLUSIONS

The no. and percentage of root tips colonised by *T. melanosporum* has increased over the ten years, especially in the most recent ones. *S. brunnea* (the only quantitatively significant contaminant) has been kept under control in recent years.

The annual quality control has allowed to monitor the progress in the nursery quality, to rapidly identify risks, limiting factors, and potential improvement of cultural practices. It served as a guarantee to both the client and the nursery. After some years, the quality control has turned into a continuous quality improvement scheme.

The main challenges for the coming years are the improvement of the plant material, the use of alternative substrates, and the introduction of molecular methods in certification.

REFERENCES Reyna S, Boronat J, Palomar E (2000) Control de calidad en la planta micorrizada con *Tuber melanosporum* Vitt. producida por viveros forestales. Revista Montes 61: 17-24. URL: www.revistamontes.net/Buscador.aspx

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