Fertilisation of *Quercus* seedlings inoculated with *Tuber melanosporum*: effects on growth and mycorrhization of two host species and two inoculation methods

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Modern truffle cultivation is based on use of inoculated seedlings, which should exhibit highly colonised roots as well as a vegetative quality enhancing field plant performance. However, poor shoot and fine root growth has been a frequent issue in inoculated *Quercus* seedlings production. Fertilisation is a common solution in forest nurseries, but high fertilisation levels have been found to inhibit the formation of ectomycorrhizas of many fungal species. The influence of slow-release fertilisation (52 mg N, 26 mg P and 36 mg K per seedling) on growth and ectomycorrhizal status of *Tuber melanosporum*-inoculated seedlings was evaluated. High quality inoculated seedlings must exhibit a root system abundantly colonised by *T. melanosporum* (Andres-Alquende et al. 2014, Murat 2015). The commercial production of these seedlings is customarily done with spore inoculum, either concentrating inoculum onto fine roots or incorporating it into substrate (Chevalier & Grente 1978, Palazón & Barriuso 2007). With the high price of sporocarps luring nurserymen into reducing inoculum application rates, selection of carrier materials providing close contact with fine roots and even distribution of inoculum is critical, especially when thousands of seedlings are produced (Averseng & Roux 2001). For *Tuber* species, little scientific information is publicly available on efficiency of the various inoculation methods or on their interaction with other nursery practices, often because of patents and confidentiality agreements (Cartié et al. 2001, Pruett et al. 2008).

The quality of inoculated seedlings is determined not only by abundance of mycorrhizas but also by vegetative quality of seedlings (Fischer & Colinas 1996). Large, nutrient-rich seedlings with high growth potential are likely to perform better in drylands with deep soils (Cortina et al. 2013), such as the agricultural lands where truffle plantations are usually established in Spain (Garcia-Barreda et al. 2007). However, problems of scarce shoot development and stunted lateral root growth have been frequent in the commercial production of *Quercus* seedlings inoculated with *T. melanosporum* (Chevalier & Grente 1978, Averseng & Roux 2001). Some inoculation methods seem to exacerbate this problem (Cartié et al. 1996, Pruett et al. 2008).

In forest nurseries a common solution for low vegetative quality of seedlings is fertilisation, which increases size, nutrient storage and root growth potential (Villar-Sal-
They were surface sterilised with a 20% so-
dium hypochlorite solution for five min-
utes, and germinated in January in a tray
with perlite and vermiculite. When seed-
lings had 6-8 leaves and had formed lateral
roots (12 weeks after seedling emergence,
in late April), they were removed from the
tray, mechanically root-pruned at the tap
top root to eliminate its defects (Palazón &
Barriuso 2007), inoculated, and transplant-
ed to Quick-pot® containers (650 ml, 18 cm
depth).

The inoculation was performed by root-
dipping, following a traditional method de-
described by Hall et al. (2007) as frequently
used in Spain. The bare roots were dipped
in a slurry of homogenised sporocarps in a
succrose solution (21% water: succrose v/w)
aimed to produce a high viscosity suspen-
sion. The spore concentration in the slurry
was adjusted to obtain an application rate
of 2.0 g fresh truffle per seedling (6-105
spores per seedling), although some vari-
ability between seedlings is impossible to
avoid due to differing levels of fine root
development. This method is compared to
a root-powdering inoculation, also concen-
trating spores onto roots but with a solid
carrier instead of a liquid one (Cartié et al.
2001). A mix of talcum powder (hydrated
magnesium silicate) and homogenised spo-
rocarps was applied onto seedling bare
roots, with spore concentration adjusted
to obtain an inoculum rate of 2.0 g fresh
truffle per seedling.

The potting substrate consisted of 12:6:1
(v/v) calcareous sandy loam soil, base-fertil-
isled Sphagnum white peat (Kekkila®
White 420 W), and limestone coarse sand.
It was solarised during summer, and subse-
quently presented a pH of 7.9, conductivity
(15) of 418 mS m⁻¹, 1590 ppm N (Kjeldahl),
32 ppm P (Olsen) and 337 ppm K (ammo-
nium acetate extraction), with pH and nu-
trient levels falling within the common range
in Spanish wild truffle soils (Garcia-
Barreda et al. 2007). A soil-based potting
mix was selected because these are still
used with good results in truffle nurseries
and research (Cartié et al. 2001, Benucci et
al. 2012). Seedlings were cultivated in a
greenhouse and sprinkle irrigated to satu-
rating 2-3 times per week during summer
and once each 7-14 days during winter.

Following the first shoot flush after inoc-
ulation (seven weeks after inoculating, in
mid June), slow-release fertiliser Osmocote
Exact Mini® (NPK 16-8-11, with a longevity
of 3-4 months at 21 °C) was added in the
surface of substrate at a dose 0.5 g L⁻¹, pro-
viding 52 mg N, 26 mg P, 36 mg K, 6.5 mg
Mg, 1.3 mg chelated Fe, 0.16 mg Mn and
0.07 mg B and Mo and 0.06 mg Zn per
root-dipping. The experiment was con-
ducted with Q. ilex as plant host.

Materials and methods

Fungal inoculum, plant material and
inoculation

The sporocarps used as inoculum were
acquired fresh and mature from planta-
tions in Sistema Ibérico mountain range
(eastern Spain). For each experiment, at
least 20 sporocarps from five plantations
were used in order to minimise spore ger-
minability differences. They were surface
sterilised with sodium hypochlorite solu-
tion, sliced thin, air dried under room con-
ditions and homogenised with a coffee
grinder (Palazón & Barriuso 2007).

We selected Q. ilex as host plant because
it is the most used species in Spanish truf-
file plantations. It was compared to Q.
fa
ginea, which also produces truffles in the
wild and is frequently used in Spanish truf-
file plantations. While having biological fea-
tures similar to Q. ilex, Q. faginea is a faster-
growing species whose nursery seedlings
show more extensive lateral roots (Silla &
Escudero 2004, Sanz-Pérez et al. 2007).
Acorns from the provenance regions Sis-
tema Ibérico (for Q. ilex) and Sistema Ibérico
Levantino (for Q. faginea) were acquired.
They were surface sterilised with a 20% so-

Results

Experiment 1: fertilisation/host species

Total dry weight of seedlings was posi-
tively affected by fertilisation (P<0.001),
with no significant differences between host
species (P=0.50 – Tab. 1). Both shoot
and root dry weight followed the same pattern, with a positive effect of fertilisation (P<0.01 in both cases) and no significant differences between host species (P=0.59 and P=0.46, respectively – Tab. 1). Stem height was positively affected by fertilisation (P<0.001), with no significant differences between host species (P=0.28 – Tab. 1). Root collar diameter was positively affected by fertilisation (P<0.01) and higher in Q. faginea than in Q. ilex (P<0.001 – Tab. 1). SRL was higher in Q. faginea (P=0.01) and in fertilised seedlings (P=0.04 – Tab. 1).

The inoculum of T. melanosporum formed mycorrhizas with all seedlings. The number of T. melanosporum mycorrhizas per seedling and the proportion of root tips colonised by T. melanosporum were higher (P<0.001 and P=0.01, respectively), with no significant effect of fertilisation (P=0.02 and P=0.04, respectively – Tab. 1). The only contaminant EM species found in seedlings was Sphaerosporella brunnea Svrek and Kubicka, showing a higher occurrence frequency on Q. ilex (P=0.02) and no significant effect of fertilisation. The distribution of T. melanosporum colonisation levels along the depth profile did not show any significant (α=0.05) interaction with fertilisation.

Experiment 2: fertilisation/inoculation method

Total dry weight of seedlings, shoot dry weight and root dry weight were higher in root-powdering inoculation than in root-dipping (P=0.004, P=0.003 and P=0.02, respectively). No significant effect of fertilisation was found (P=0.08, P=0.14 and P=0.08, respectively), although the trend with respect to fertilisation was the same as in the fertilisation/host species experiment (Tab. 2). Stem height was not significantly affected by either fertilisation (P=0.28) or inoculation method.
inoculation method (P=0.26 – Tab. 2). Root collar diameter was positively affected by fertilisation (P=0.02), with no significant differences between inoculation methods (P=0.16 – Tab. 2). Neither SRL nor number of root tips per seedling were significantly affected by fertilisation (P=0.54 and P=0.39, respectively) or inoculation method (P=0.59 and P=0.12, respectively – Tab. 2).

The inoculum of T. melanosporum formed mycorrhizas with all seedlings. The number of T. melanosporum mycorrhizas per seedling, and the proportion of root tips colonised by T. melanosporum were higher in root-powdering inoculation (P=0.009 and P=0.049, respectively), with no significant effect of fertilisation (P=0.46 and P=0.68, respectively – Tab. 2). The mean proportion of root tips colonised by S. brunnea in root-dipping inoculation was 5.2%.

The distribution of T. melanosporum colonisation levels along the depth profile did not show any significant (α=0.05) interaction with fertilisation.

Discussion

In modern truffle cultivation the use of inoculated seedlings is fundamental (Hall et al. 2007). The abundance of T. melanosporum mycorrhizas in the early years after plantation establishment has been found positively related to mycorrhizal abundance in the nursery and to plant performance after planting (Bournières et al. 2005, García-Barreda & Reyna 2015). Nursery practices must be fine-tuned to encourage colonisation by T. melanosporum, but also to improve vegetative quality of seedlings. However, in nursery fertilisation experiments a conflict between optimal seedling growth and EM colonisation has been reported for many EM fungi (Castellano & Molina 1989, Walker et al. 2003, Díaz et al. 2010). Two mechanisms have been suggested to explain this conflict: (i) the host reducing carbon supply to the fungus due to a greater carbon demand by growing shoots, or (ii) the fungus requiring most carbon received from the plant to assimilation of the greater N uptake (Wallander 1995).

The fertilisation dose used in the present study increased growth as well as vegetative quality levels along the depth profile did not show any significant (α=0.05) interaction with fertilisation. The fertilisation dose used in the present study increased growth as well as vegetative quality levels along the depth profile did not show any significant (α=0.05) interaction with fertilisation.

In our study, Q. ilex and Q. faginea showed very similar biomass and response to fertilisation, as expected for species with similar biological features and agreeing with results of Sanz-Pérez et al. (2007) for non-inoculated seedlings fertilised with 50 mg N. We found the main differences between these species in fine root traits and EM status, with Q. faginea showing higher SRL, number of root tips and number of mycorrhizas. This agrees with findings of Dominguez-Núñez et al. (2006) and Silla & Escudero (2004) in young plantations. The former found more root tips and EM tips in Q. faginea than in Q. ilex seedlings inoculated with T. melanosporum, whereas the latter found higher SRL in Q. faginea than in Q. ilex non-inoculated seedlings. Our results agree with the accepted view that Q. faginea root system is more branched and extensive. The distinct fine root traits of Q. ilex and Q. faginea went along with differences in EM status. This could be due to the inoculation method delivering somewhat more spore inoculum to seedlings with higher SRL, to inoculum being more evenly distributed within seedling fine roots, or to seedlings recovering before from transplanting practices. However not only the inoculation and then powdering them with a mixture of inoculum and talcum. Pruett et al. (2008) found that adding hydrogel to a water slurry for root-dipping inoculation had negative effects on Quercus robur L. survival, root development and Tuber aestivum Vittad. colonisation. Cartié et al. (1996) found that using an alginate solution for root-dipping inoculation provoked an important Q. ilex mortality and a low T. melanosporum colonisation. These results suggest that inoculant carriers forming a sticky coating around the complete root system are able to damage Quercus development and Tuber colonisation.

However, Cartié et al. (2001) found no detrimental effects of alginate solution when the inoculant was applied in a bilayer, firstly dipping roots in alginate solution and then powdering them with a mixture of inoculum and talcum. Pruett et al. (2008) found no detrimental effect of root-dipping inoculation when it was performed without hydrogel. All this suggests that the damage was due to the combination of sticky carrier and spores in close contact with roots.

Our results show that seedling characteristics and inoculation effectiveness can be affected not only by host species but also by inoculation method. Further research would help to know if fertilisation or inoculation method interact with other nursery practices.

The only EM fungi found in our study other than T. melanosporum was the pioneer, nursery adapted S. brunnea (Sánchez et al. 2014). As expected, its occurrence was higher in treatments with lower T. melanosporum colonisation levels, thus suggesting that it was related to gaps left by the latter.

The inoculation and fertilisation procedures used in the present study proved effective for obtaining EM seedlings with quality levels comparable to commercial standards. All seedlings bore T. melanosporum mycorrhizas, with all treatments showing mean colonisation levels analogous to those in commercial nurseries (Andres-Alpuente et al. 2014). All seedlings met the

Conclusions

This study showed that a dose of slow-release fertiliser is able to improve growth and morphological quality of Quercus seedlings while maintaining commercial T. melanosporum colonisation levels under greenhouse conditions. Quercus ilex and Q. faginea showed differences mainly in fine root traits and EM status, but in spite of them both species showed similar response to the fertilisation dose. The inoculation method proved able to influence not only EM status but also seedling growth. The study provided an important basis for fine-tuning use of fertilisation in commercial production of T. melanosporum inoculated seedlings, but showed that inoculation effectiveness can be altered by other cultural practices, hence the improvement of these practices should be addressed jointly.

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References


