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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. Host species *Quercus ilex* and *Quercus faginea* and inoculation methods involving root-dipping and root-powdering were tested. Fertilisation increased weight of both host species without significant detrimental effects on ectomycorrhizal colonisation, showing that it can be effectively used in inoculated seedlings production. Both host species showed similar response to fertilisation. The inoculation method affected seedling weight and ectomycorrhizal status, suggesting that some inoculant carriers are able to damage *Quercus* development and *T. melanosporum* colonisation. The study provided an important basis for fine-tuning the use of fertilisers in truffle-inoculated seedling production.

Keywords: Fertilisation, Seedling, Nursery, Ectomycorrhizal, Inoculation

Introduction

The European black truffle (*Tuber melanosporum* Vittad.) is an ectomycorrhizal (EM) fungus extensively cultivated due to its gastronomic value and decline of wild production (Reyna & Garcia-Barreda 2014). Each year, more than 2,000 ha of agricultural lands are afforested in southern Europe (mainly France, Italy and Spain) by entrepreneurs to produce black truffle, most of them with native *Quercus* as host plants (Reyna & Garcia-Barreda 2014). Modern truffle cultivation is based on planting nursery-inoculated seedlings on suitable lands, with appropriate edaphoclimatic environment for the fungus to complete its life cycle and with low EM inoculum potential (Sourzat 2008).

High quality inoculated seedlings must exhibit a root system abundantly colonised by *T. melanosporum* (Andres-Alpuente 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 & Rouch 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 & Rouch 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-

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vador et al. 2004). However, Treseder (2004) showed that nitrogen (N) and phosphorus (P) fertilisations decrease mycorrhizal abundance in the field. In nursery experiments, high fertilisation levels have been found to inhibit the formation of ectomycorrhizas of many fungal species including *T. melanosporum* (Dupré et al. 1982, Beckjord et al. 1985, Walker et al. 2003, Diaz et al. 2010), although Castellano & Molina (1989) noticed that some species are much more tolerant and that fertilisation effect was dependent on the application system, fertiliser type, dose and form. On the other side, Quoreshi & Timmer (2000) and Rincon et al. (2007) found fertiliser doses that increased growth of containerised conifer seedling without decreasing colonisation levels of inoculated fungi from various EM species.

In this study we inoculated *Quercus ilex* L. and *Quercus faginea* Lam. with *T. melanosporum*. We hypothesised that a moderate fertilisation regime could improve morphological quality of seedlings while maintaining EM colonisation levels suitable for commercial purposes. We assess the effect of fertilisation on growth and EM status of seedlings, applying a dose similar to that used by Rincon et al. (2007). We compare the effect of fertilisation on *Q. ilex* and *Q. faginea*, a faster-growing species (Sanz-Pérez et al. 2007), and we evaluate the use of fertilisation in two inoculation methods, root-dipping and root-powdering, both applying inoculum onto roots, although with different carrier materials. We hypothesised that host species and inoculation method would affect fine root traits, thus influencing EM status and ability of seedlings to respond to fertilisation.

Materials and methods

Fungal inoculum, plant material and inoculation

The sporocarps used as inoculum were acquired fresh and mature from plantations in *Sistema Ibérico* mountain range (eastern Spain). For each experiment, at least 20 sporocarps from five plantations were used in order to minimise spore germinability differences. They were surface sterilised with sodium hypochlorite solution, sliced thin, air dried under room conditions 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 truffle plantations. It was compared to *Q. faginea*, which also produces truffles in the wild and is frequently used in Spanish truffle plantations. While having biological features 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 *Sistema Ibérico* (for *Q. ilex*) and *Sistema Ibérico Levantino* (for *Q. faginea*) were acquired. They were surface sterilised with a 20% sodium hypochlorite solution for five minutes, and germinated in January in a tray with perlite and vermiculite. When seedlings 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 root end to eliminate its defects (Palazón & Barriuso 2007), inoculated, and transplanted to Quick-pot® containers (650 ml, 18 cm deep).

The inoculation was performed by root-dipping, following a traditional method described by Hall et al. (2007) as frequently used in Spain. The bare roots were dipped in a slurry of homogenised sporocarps in a sucrose solution (2:1 water: sucrose v/w) aimed to produce a high viscosity suspension. The spore concentration in the slurry was adjusted to obtain an application rate of 2.0 g fresh truffle per seedling (6×10^5 spores per seedling), although some variability between seedlings is impossible to avoid due to differing levels of fine root development. This method is compared to a root-powdering inoculation, also concentrating 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 sporocarps 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-fertilised *Sphagnum* white peat (Kekkila® White 420 W), and limestone coarse sand. It was solarised during summer, and subsequently presented a pH of 7.9, conductivity (1:5) of 418 mS m^{-1}, 1390 ppm N (Kjeldahl), 32 ppm P (Olsen) and 337 ppm K (ammonium acetate extraction), with pH and nutrient 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 saturation 2-3 times per week during summer and once each 7-14 days during winter.

Following the first shoot flush after inoculation (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}, providing 52 mg N, 26 mg P, 36 mg K, 6.5 mg Mg, 1.3 mg chelated Fe, 0.16 mg Mn and Cu, 0.07 mg B and Mo and 0.06 mg Zn per seedling. We selected a slow-release fertiliser because it is the most common in Spanish forest nurseries. The dose was selected following that used by Rincon et al. (2007) for avoiding inhibition of EM formation in containerised *Pinus* seedlings.

Experimental design

Two experiments were done independently. In 2009 an experiment to compare the effect of fertilisation on two host species (*Q. ilex* and *Q. faginea*) was conducted. Four treatments were established in a 2×2 factorial design, with 12 replicates per treatment: unfertilised *Q. ilex*, fertilised *Q. ilex*, unfertilised *Q. faginea* and fertilised *Q. faginea*. All seedlings were inoculated with root-dipping.

In 2010 an experiment to compare the effect of fertilisation on two inoculation methods was conducted. Four treatments were established in a 2×2 factorial design, with six replicates per treatment: unfertilised root-dipping, fertilised root-dipping, unfertilised root-powdering and fertilised root-powdering. The experiment was conducted with *Q. ilex* as plant host.

Data collection and analysis

The seedlings of the fertilisation and host species experiment were analysed 12 months after inoculation (in April-May), whereas the fertilisation and inoculation method experiment was analysed 11 months after inoculation (in March). The mycorrhizal status was assessed through a volumetric sampling. In each seedling a sample with 9% of the substrate volume (57 ml) was taken. To cope with heterogeneity across soil depth, every sample consisted of three subsamples: the depth of the container was divided into three equal parts, and in the centre of each third (4, 9 and 13.5 cm depth) a horizontal core (2 cm diameter) across the container was taken.

Samples were kept in water at 4°C. Length of fine roots (diameter <2 mm) was measured according to Tennant (1975). Root tips were counted and classified as non-mycorrhizal, *T. melanosporum* ectomycorrhizas or contaminant ectomycorrhizas. Shoot dry weight, root dry weight, fine root dry weight, stem height and root collar diameter were measured after drying to constant weight at 80 °C.

Plant dry weight, shoot and root dry weight, stem height, root collar diameter, specific root length (SRL, ratio of root length to dry weight of fine roots), number of root tips and number of *T. melanosporum* tips per seedling were analysed by conventional ANOVA. Proportion of root tips colonised by *T. melanosporum* and frequency of occurrence of contaminants in seedlings were analysed through generalised (binomial) linear models. When model assumptions were violated, the response variable was transformed. In the model for proportion of roots colonised by *T. melanosporum*, fine root length was included as a covariate to account for within-treatment variability in fine root development.

Results

Experiment 1: fertilisation/host species

Total dry weight of seedlings was positively affected by fertilisation (P<0.001), with no significant differences between host species (P=0.50 – Tab. 1). Both shoot

Tab. 1 - Mean growth and mycorrhizal levels in Experiment 1: fertilisation/host species (95% confidence interval in parentheses). Letters indicate significant differences (α=0.05) between treatments according to the respective ANOVA or linear model. (§): Variables log-transformed.

Variable	Host species		Fertilisation regime	
	Q. ilex	*Q. faginea*	Unfertilised	Fertilised
Plant dry weight (g)	5.34 (4.79, 5.90)	5.61 (5.05, 6.17)	4.06 [j] (3.51, 4.62)	6.89 [i] (6.34, 7.45)
Shoot dry weight (g) [§]	1.51 (1.32, 1.75)	1.44 (1.25, 1.66)	1.02 [j] (0.89, 1.17)	2.14 [i] (1.86, 2.46)
Root dry weight (g) [§]	3.50 (3.13, 3.91)	3.71 (3.32, 4.14)	2.88 [j] (2.57, 3.21)	4.50 [i] (4.03, 5.03)
Stem height (cm)	15.8 (13.9, 17.7)	15.3 (13.4, 17.1)	12.5 [j] (10.6, 14.4)	18.6 [i] (16.7, 20.5)
Root collar diameter (mm)	6.1 [b] (5.7, 6.6)	7.9 [a] (7.5, 8.4)	6.3 [j] (5.9, 6.8)	7.7 [i] (7.2, 8.1)
Specific root length (m g^{-1}) [§]	8.4 [b] (7.2, 9.8)	12.3 [a] (10.6, 14.3)	10.9 (9.3, 12.7)	9.5 (8.2, 11.1)
Number of root tips (×10^3) [§]	4.20 [b] (3.37, 5.24)	10.04 [a] (8.05, 12.53)	5.52 [j] (4.42, 6.89)	7.64 [i] (6.12, 9.53)
Number of mycorrhizas *T. melanosporum* (×10^3) [§]	1.28 [b] (0.97, 1.68)	4.62 [a] (3.52, 6.06)	2.31 (1.76, 3.03)	2.56 (1.95, 3.36)
Percentage of roots colonised by *T. melanosporum*	35.0 [b] (34.1, 35.9)	45.4 [a] (44.5, 46.4)	42.1 (39.8, 44.4)	38.3 (36.1, 40.6)
Frequency of occurrence of *S. brunnea*	0.17 [a] (0.01, 0.33)	0 [b]	0.04 (0, 0.13)	0.13 (0, 0.27)

and root dry weight followed the same pattern, with a positive effect of fertilisation (P<0.001 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=0.69 – Tab. 1). Root collar diameter was positively affected by fertilisation (P<0.001) and higher in *Q. faginea* than in *Q. ilex* (P<0.001 – Tab. 1). SRL was higher in *Q. faginea* (P=0.001), with no significant effect of fertilisation (P=0.22 – Tab. 1). The number of root tips per seedling was higher in *Q. faginea* (P<0.001) 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 in *Q. faginea* (P<0.001 and P=0.01, respectively), with no significant effect of fertilisation (P=0.59 and P=0.24, respectively – Tab. 1). The only contaminant EM species found in seedlings was *Sphaerosporella brunnea* Svrcek and Kubicka, showing a higher occurrence frequency on *Q. ilex* (P=0.02) and no significant effect of fertilisation (P=0.22 – Tab. 1). The mean proportion of root tips colonised by *S. brunnea* on *Q. ilex* was 2.5%.

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

Tab. 2 - Mean growth and mycorrhizal levels in Experiment 2: fertilisation/inoculation method (95% confidence interval in parentheses). Letters indicate significant differences (α=0.05) between treatments according to the respective ANOVA or linear model. (§): Variables log-transformed.

Variable	Inoculation method		Fertilisation regime	
	Root-dipping	Root-powdering	Unfertilised	Fertilised
Plant dry weight (g) [§]	3.54 [b] (3.09, 4.05)	4.73 [a] (4.14, 5.41)	3.76 (3.29, 4.30)	4.45 (3.89, 5.09)
Shoot dry weight (g) [§]	1.19 [b] (1.01, 1.40)	1.72 [a] (1.46. 2.02)	1.31 (1.11, 1.54)	1.56 (1.32, 1.83)
Root dry weight (g) [§]	2.33 [b] (2.03, 2.68)	2.99 [a] (2.60, 3.44)	2.42 (2.10, 2.78)	2.88 (2.51, 3.31)
Stem height (cm)	14.8 (12.0, 17.5)	16.9 (14.2, 19.6)	14.8 (12.1, 17.5)	16.9 (14.1, 19.6)
Root collar diameter (mm)	5.7 (5.3, 6.1)	6.1 (5.7, 6.5)	5.6 [j] (5.1, 6.0)	6.3 [i] (5.9, 6.7)
Specific root length (m g^{-1})	23.6 (20.9, 26.2)	22.6 (19.9, 25.2)	23.6 (21.0, 26.3)	22.5 (19.9, 25.2)
Number of root tips (×10^3) [§]	4.84 (3.49, 6.69)	6.92 (5.00, 9.57)	6.37 (4.60, 8.81)	5.25 (3.80, 7.27)
Number of mycorrhizas *T. melanosporum* (×10^3) [§]	1.62 [b] (1.24, 2.12)	2.74 [a] (2.10, 3.58)	2.26 (1.73, 2.95)	1.97 (1.51, 2.57)
Percentage of roots colonised by *T. melanosporum*	32.6 [b] (29.7, 35.5)	43.7 [a] (37.8, 49.5)	37.6 (31.6, 43.5)	38.6 (35.0, 42.2)
Frequency of occurrence of *S. brunnea*	0.42 [a] (0.12, 0.71)	0 [b]	0.25 (0, 0.51)	0.17 (0, 0.39)

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 only contaminant EM species found was *S. brunnea*, showing a higher occurrence frequency in root-dipping inoculation (P=0.004) and no significant effect of fertilisation (P=0.56 – 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 (Bourrières et al. 2005, Garcia-Barreda & Reyna 2013). 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, Diaz 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 according to Spanish standards (Villar-Salvador et al. 2012a, 2012b), while maintaining *T. melanosporum* colonisation levels. In the fertilisation/host species experiment, fertilisation increased seedling biomass by 70%, stem height by 49% and root collar diameter by 22%, without significant detrimental effects on fine root traits or EM colonisation levels. In the fertilisation/inoculation method experiment, fertilisation increased *Q. ilex* root collar diameter by 13%. The lower magnitude of the fertilisation effect in the latter experiment could be due to differences in acorn size (Navarro et al. 2006), spore germinability (Palazón & Barriuso 2007) or greenhouse climatic conditions from year to year, although it could also be related to the lower number of replicates decreasing the accuracy of estimation.

Our results disagree with previous experiments with containerised *Quercus* seedlings. Beckjord et al. (1985) inoculated *Quercus alba* L. and *Quercus rubra* L. with *Pisolithus tinctorius* (Pers.) Coker & Couch and *Scleroderma citrinum* Pers. and found that N and P fertilisation doses suitable for mycorrhizal colonisation were much lower than those needed to promote seedling growth. Dupré et al. (1982) inoculated *Quercus humilis* Mill. with *T. melanosporum* and found that P fertilisation doses with increased seedling growth did not correspond with higher mycorrhizal colonisation. In *Q. ilex* inoculated with *Paxillus involutus* (Batsch) Fr., *Hebeloma mesophaeum* (Pers.) Quél. and *Cenococcum geophilum* Fr., Oliveira et al. (2010) found that a fertiliser dose moderately higher than ours decreased mycorrhizal colonisation, with no significant increase in plant biomass or shoot height. On the other hand, Rincon et al. (2007), Quoreshi & Timmer (2000) and Khasa et al. (2001) found fertilisation levels that improved conifer growth without damaging colonisation levels by various inoculated EM fungi, supporting that at least in some cases they can be reconciled.

The contrast between our results and other *Quercus* experiments could be due to differences in sensitiveness of EM species to fertilisers (Castellano & Molina 1989, Wallander 1995), differences in the response of host species to fertilisation, differences in fertilisation implementation or differences in other cultivation practices. The response of non-inoculated *Q. ilex* seedlings to fertilisation follows a pattern similar to other Mediterranean *Quercus* such as *Q. faginea*, but different from *Quercus* from other biomes which show lower sufficiency levels (Uscola et al. 2015). Castellano & Molina (1989) warned that response of EM fungi to fertilisation was influenced not only by the dose but also by fertiliser type and form, as well as application system (timing, rate and method).

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 Domínguez-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 transplantation. However no interaction in the response of fine root traits to fertilisation was found, suggesting that differences between *Q. ilex* and *Q. faginea* did not affect ability of seedlings to respond to fertilisation.

The inoculation method influenced seedling weight and EM status, with root-dipping showing worse characteristics. Pruett et al. (2008) found that adding hydrogel in 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 bi-layer, 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

morphology standards established in relevant regulation on forest seedlings marketing (European Directive 1999/105/CE for *Q. ilex* and Spanish RD 289/2003 for *Q. faginea*).

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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