Düzce University Faculty of Forestry
Journal of Forestry
(DUJOF)

Journal of Forestry Volume 18, Number 2, pp. 405-420
Category: Research Article

https://dergipark.org.tr/tr/pub/duzceod
ISSN 2148-7855 (online), ISSN 2148-7871
Düzce University Faculty of Forestry

Comparison of Mycorrhizal Colonization Success in Oak Species Inoculated with *Tuber aestivum* Vitt. and *Tuber borchii* Vitt.*

Tuber aestivum Vit. ve *Tuber borchii* Vit. ile İnokule Edilen Meşe Fidanlarının Mikorizal Kolonizasyon Başarısının Karşılaştırılması

Refika Ceyda BERAM[1], Murat MAHSUN[2], H. Tuğba DOĞMUŞ LEHTİJÄRVİ[2]

Abstract

Truffles are ascomycete ectomycorrhizal fungi of the genus *Tuber*. Although edible fungi in this genus very high economic values, they occur naturally in limited quantities and in restricted geographical areas of the world. Nowadays, more than half of the truffles used commercially are harvested truffiere (truffle orchards). The quality of the truffle inoculated seedlings and the edaphic conditions in cultivation areas are the most important factors for truffle production. *Tuber aestivum* (summer truffle) and *T. borchii* (bianchetto truffle) are the leading truffle species with high commercial value in Türkiye and are collected by truffle hunters from their natural distribution areas. In this study, mycorrhizal colonization successes of *T. aestivum* and *T. borchii* species were investigated in *Q. coccifera* and *Q. robur* seedlings. Oak seeds collected from natural stands were germinated, inoculated with a spore-based truffle inoculation technique and incubated in a greenhouse located in Denizli Karahasanlı Forest Nursery. The results of this work revealed that both *T. borchii* and *T. aestivum* spores produced well-formed ectomycorrhizae on seedlings. According to certification standards, *Q. robur* is the oak species with a suitable mycorrhizal growth rate for both *Tuber* species. The presence of inoculum had a positive effect on both seedling species.

Keywords: Mycorrhiza, Truffle inoculation, *Tuber* sp., Denizli, Oak.

Özet

Tuber cinsinin askomiset ektomikorizal üyeleri türüf mantarları olarak bilinmektedir. Bu cinse ait yenilebilir mantarlar ekonomik olarak çok değerli olmasının yanı sıra dünyada sınırlı coğrafi bölgede kısıtlı miktarlarda yetişmektedir. Günümüzde dünyada elde edilen trüf miktarının yarısından fazlası trüf bahçelerinden hasat edilmektedir. Trüf aşılı fidanların kalitesi ve kültivasyon alanlarının edafitik koşulları bu fungusların askokarp üretmesi için en önemli faktörlerdir. *T. aestivum* ve *T. borchii*, Türkiye'de ticari değeri yüksek olan ve trüf avcıları tarafından doğal yayılış alanlarından toplanan trüf türlerinin başında gelmektedir. Bu çalışmada, *Q. coccifera* ve *Q. robur* fidanlarında *T. aestivum* ve *T. borchii* türlerinin mikorizal kolonizasyon başarıları araştırılmıştır. Doğal alanlardan toplanan meşe tohumları çimlendirilmiş, spor bazlı inokulasyon tekniği ile aşılanmış ve Denizli Karahasanlı Orman Fidanlığı'nda bulunan serada inkubasyona bırakılmıştır. Bu çalışmanın sonuçları, hem *T. borchii* hem de *T. aestivum* sporlarının fidanlar üzerinde başarılı ektomikorizalar ürettiğini ortaya koymuştur. Sertifikasyon standartlarına göre *Q. robur* başarılı mikorizal kolonizasyon oranına sahip meşe türüdür. İnokulum varlığının her iki fidan türü üzerinde olumlu etkisi olduğu tespit edilmiştir.

Anahtar Kelimeler: Mikoriza, Türüf inokulasyonu, *Tuber* sp., Denizli, Meşe.

Received: 27.11.2022, Revised: 26.12.2022, Accepted: 14.12.2022
Address: [1]Pamukkale University, Faculty of Science, Department of Biology, Denizli, Türkiye
Address: [2] Isparta University of Applied Sciences, Faculty of Forestry, Department of Forest Engineering, Isparta, Türkiye
E-mail: rberam@pau.edu.tr
*This study is prepared based on master thesis of second author at.

1. Introduction

Ectomycorrhizal fungi, living in symbiosis with a wide range of trees and shrubs, and widely distributed across temperate regions in the Northern Hemisphere, play important roles in forest functioning and biogeochemical cycles (Berch and Bonito, 2016). They are also responsible for a significant portion of forest-soil carbon flux. The mycorrhizal association helps plants to absorb nutrients from the soil, protects the roots from pathogens and decomposes organic matter. Apart from forest ecosystems, ectomycorrhizal trees are used in agroforestry. Ectomycorrhizal fungi constitute an economically important mycorrhizal association. Some important types of mycorrhizal fungi produce edible (hypogeous fruiting bodies) fructifications with high commercial values. representing income opportunities for farmers and foresters. These fungi have been grown successfully in laboratory conditions and some species used in inoculations of tree seedlings (Smith and Read, 2008).

The 'mantle' and 'Hartig net' distinguishes ectomycorrhizae from arbuscular mycorrhizae. Hyphae contact the young, unsuberized growing roots and begin the superficial infection. Root exudates attract the propagules to the plant tissues and stimulate rapid growth, meaning that root tips are quickly covered with a dense sheath of hyphae (fungal-mantle) (Mukerji et al., 2000). In addition, mycorrhizal roots can be used as an identification tool due to the presence of the Hartig net and mantle, as they are thicker than other fine roots. Formation of mycorrhizal infections and their distribution are amongst the most important parameters to determine in terms of the function of mycorrhiza (Ortaş, 1998; Fischer and Colinas, 1996).

While most known ectomycorrhizal fungi are Basidiomycota (Agaricomycetes), such as *Boletus, Suillus, Russula, Hebeloma, Tricholoma, Laccaria, Rhizopogon, Scleroderma, Alpova, Pisolithus* and many others, some of Ascomycota, such as *Tuber* and *Cenococcum*, are also ectomycorrhizal (Smith and Read, 2008). Truffles are Ascomycota ectomycorrhiza-forming species, mainly in the genus *Tuber* (about 200 species), including commercially important European species such as *Tuber magnatum* Picco (Italian white truffle), *T. melanosporum* Vitt. (black truffle), *T. aestivum* (summer truffle), and *T. borchii* Vitt. (bianchetto truffle) (Bonito et al., 2010; Leonardi et al., 2020).

The winter fruiting species *T. magnatum* and *T. melanosporum* are naturally restricted to limited areas of southern Europe. Other species, such as *T. aestivum* and *T. borchii*, are found throughout Europe (Gryndler et al., 2011). Moreover, cultivation of *T. Aestivum, T.*

borchii and *T. melanosporum* is possible in some countries where suitable conditions exist, such as Italy, France, New Zealand and the United States (Zambonelli et al., 2002).

Decreasing amounts of truffles are found in natural environments each season, resulting in an increase in demand for truffles. This situation has the led to a demand for the establishment of truffiere. Truffle cultivation is a long-term process that requires patience. First truffle harvest in these cultivations may take 5 years or more, although it was reported that the first truffle emerged in ideal conditions in much less time. Selection of high quality host plants is an important component in obtaining early yields. An important practical facet in truffle cultivation is the successful inoculation of host seedlings. The necessity of ectomycorrhizal inoculation for successful reforestation was first introduced by Kessell (1927) in Australia in 1927. Nowadays, more than half the annual truffle quantities obtained globally is harvested from truffiere (Mello et al., 2006).

Türkiye has a rich biodiversity due to its location and climate, and is highly productive in terms of ectomycorrhizal fungi, including truffles. *Tuber aestivum* and *T. borchii* are valuable truffle species with high commercial value in Türkiye and are collected by truffle hunters from their natural distribution areas (Türkoğlu, 2015). Although studies on truffles in Türkiye were limited in the past, today the interest in truffles is increasing. Along with the Truffle Forest Action Plan instigated by the General Directorate of Forestry in Türkiye (Anon.,, 2014), truffle research has gained great momentum and studies concerning identification and protection of natural truffle areas and the establishment of artificial truffiere have increased. Within the scope of the action plan, truffle-inoculated seedlings are produced in forest nurseries in Denizli, Mula, Lüleburgaz, Eskişehir and Samsun. The production and trade in truffle-inoculated seedlings has become an important commercial concern. Due to the importance of truffles in the food market, they are an important source of income for rural development, tourism and other sectors, contributing to the rural economy. In addition, the positive benefits of mycorrhizal fungi on plant growth make them indispensable elements of nursery and afforestation programmes.

Ectomycorrhizal fungi are most common in the tree families Pinaceae, Salicaceae, Betulaceae, Fagaceae and Tiliaceae, as well as members of the Rosaceae, Leguminaceae, Myrtaceae and Juglandaceae. In Türkiye, species of truffles appears to be abundant in oaks ectomycorrhizal (EM) communities. Oak is the common name given to the genus *Quercus* L. and there are 17 species and 23 taxa of this genus in Türkiye (Akkemik et al., 2019). When the forests of Türkiye are examined in terms of tree species and the area they cover, oaks occupy the first place with a distribution area of 6.7 million hectares (Anon., 2020). Within

the scope of this research, mycorrhizal colonization success of the highly valuable European truffle species (*T. aestivum* and *T. borchii*) on *Quercus robur* L. and *Quercus coccifera* L. seedlings was investigated and compared.

2. Materials and Methods

2.1. Materials

2.1.1. Study Area

This work was carried out in Denizli Karahasanlı Forest Nursery (Figure 1), affiliated to Denizli Regional Directorate of Forestry. The nursery is at an altitude of 450 meters and faces northwest. According to the 30-year (between 1987-2016) observation averages of Denizli; Annual average temperature 16.58 °C, annual high temperature average 22.9 °C, highest temperature 44.4 °C, annual low temperature average 11.4 °C, lowest temperature -10.5 °C, total annual precipitation average 45.1 mm, long years, the maximum precipitation is 105.6 mm, the annual average relative humidity is 58.6 %, the lowest relative humidity is 24.5 %. According to the Thornthwaite (1948) climate classification, the region where the nursery is located is in the C1 semi-arid, less humid climate class. For many years, the hottest months according to the daily average temperature values were July and August.

Figure 1. Study area.

2.1.2. Greenhouse Conditions

The greenhouse used in the study (Figure 2) was 30 m long, 7.5 m wide, enclosing an area of 225 m^2. The height at the apex was 3 m. It was a single-cell geotic structure covered with 4-chamber polycarbonate sheets of 10 mm thickness. Greenhouse ventilation was via 2 exhaust fans with a flow rate of 18.000 m^3 / hour, with opening and closing of the top covers

by means of an automated system depending on the internal temperature. Cooling was provided by an evaporative cooler with a flow rate of 36.000 m^3/hour.

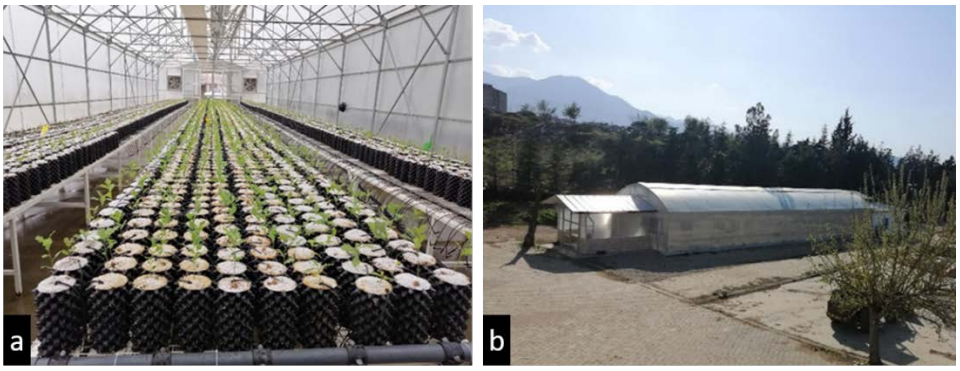


Figure 2. Greenhouse used in the study a) the internal view of the greenhouse b) the extarnal view of the greenhouse.

2.1.3. Irrigation Properties

Based on previous analyses, the irrigation water was in the C2-S1 class: clear, medium salty, low sodium and calcium bicarbonate water, pH 7.97. An ultraviolet water treatment system was installed to sterilize the irrigation water used in the greenhouse and its outbuildings.

2.1.4. Plants

Seeds of common (*Q. robur*) and Kermes oak (*Q. coccifera*), were collected in the ripening periods (November-December, 2018) in Denizli-Türkiye. *Quercus robur* seeds were collected from the trees around Pamukkale University in Pamukkale district of Denizli and *Q. coccifera* seeds were collected from trees around Çakmak-Saruhanlı cemetery in the Merkezefendi district of Denizli.

Seeds with cracks, holes and those smaller than normal were discarded. Selected healthy seeds were first washed in tap water and surface sterilized with bleach for 30 minutes. Following rinsing in sterile distilled water and drying on paper towels, seeds were stored in perlite in plastic containers at +4 °C until the inoculation.

2.1.5. Mycorrhizal Fungi

Tuber aestivum ascocarps were collected from naturally infected *Quercus* in June 2018 in Çivril district of Denizli; *T. borchii* ascocarps were collected from natural *Quercus* sp. areas located around in Buldan district of Denizli province with the help of truffle dogs. The soil on the ascocarps was cleaned off and any ascocarps with rotten parts discarded. The selected ascocarps were sterilized again by dipping in 70% ethanol and and flame sterilized

before storing in plastic bags at -20°C until the inoculation experiments were initiated (Giorgio et al., 2016; Yuanzhi, 2016).

Fresh ascomata were examined for macromorphological characteristics such as color, shape, and size of peridium and gleba. Microscopic observations were performed in distilled water, using Melzer's reagent (Langeron and Vanbreuseghem, 1952).

2.1.6. Growing Media and Containers

Peat, vermiculite and perlite were used as growing media. Growth medium materials were placed in autoclave bags and sterilized by autoclaving twice for 60 minutes at 121°C 1.5 atm pressure. Enso pots with 45 chambers were used in the production of seedlings. The diameter of the bottom circle of one chamber of enso pots was 3.5 cm, the diameter of the bottom circle was 5 cm, and the depth was 16 cm. The pot volume was 0.23 liters. The bottom of the pots is designed open to drain water and prevent root curling. All pots to be used were surface sterilized and kept in ultraviolet light before use.

2.2. Inoculations

Each fungus (*T. aestivum* and *T. borchii*) was inoculated onto both *Q. robur* and *Q. coccifera* with 45 replicates per species. In addition, 45 *Q. robur* and 45 *Q. coccifera* were grown as controls in a growing medium without fungal inoculation.

2.2.1. Germination of Seeds

For germination, 300 seeds of each oak species were taken to the greenhouse in February. Sterile perlite and sterile seeds were moistened by adding sterile distilled water and maintained in a humid environment at 20°C, 50-60% humidity in the greenhouse to germinate (Figure 3a). After two weeks, seeds were subsequently checked daily and germinated seeds counted and recorded. Seedlings with suitable criteria for truffle inoculation were selected and stored until inoculation (Fischer and Colinas, 1996; Council Directive 1999/105/EC of 22 December, 1999).

2.2.2. Preparation of Growth Media and Inoculation

Three gr of ascocarp was used for each seedling. Ascocarps were crushed and blended into a paste in a blender. Mycorrhizal mixture was prepared by adding 10 grams of agar to a liter of water and boiling, cooling and mixing the pulp from the blender into the resulting jellyfied water (Figure 3b). The suspension was mixed carefully with 3 l of vermiculite to a homogeneously mixture in the peat. A further 3 l of perlite was added to this mixture in order

to increase the air and water holding capacity of the growing medium, with mixing to homogeneity.

Tuber aestivum spore-vermiculite-perlite mixture obtained with 12 liters of peat for ninety seedlings was mixed until homogeneous. The final growing medium was placed in the Enso spots and a germinated seed was planted in each well. Perlite was added again on the enso pot in order not to lose the moisture of the sown seeds. After planting process, pots were placed on benches in the greenhouse and maintained as described below.

The same procedures were applied for plants inoculated with *T. borchii*. No mycorrhizal mixture was applied to control seedlings. The pots containing seeds were placed on benches in the greenhouse for regular irrigation and maintenance each day. The seedlings were left to grow for a vegetation period at 50 % humidity, 12 hour daylight and at 25-35 °C (Zambonelli et al.,1993).

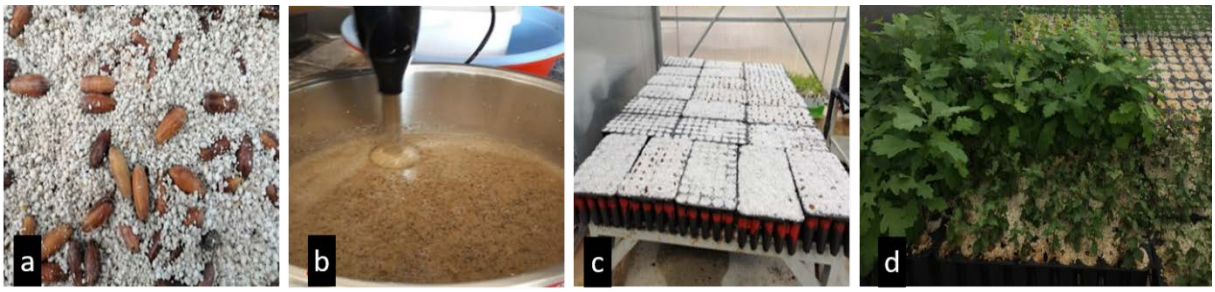


Figure 3. Inoculation steps a) germination of seeds in perlite b) preparation of mycorrhizal inoculum c) pots on the greenhouse bench after inoculation d) incubation in greenhouse.

2.2.3. Determination of Mycorrhizal Colonization Rate

At the end of a vegetation period, mycorrhiza-inoculated and control seedlings were randomly selected (10 of each) and transported to the laboratory to assess and quantify mycorrhizal colonization (Fischer and Colinas, 1996). Seedlings were gently removed from the Enso pots and the root systems dipped in water for half an hour to soften the growing medium around the root, washing to remove the peat. Root collar diameters were measured with calipers (Figure 4a), root and stem lengths measured with a ruler, and root and stem fresh weights obtained (Figure 4b). Then 2 cm pieces were cut from the roots and placed in Petri dishes in distilled water (Fischer and Colinas, 1996; Reyna et al., 2000; Avis et al., 2003).

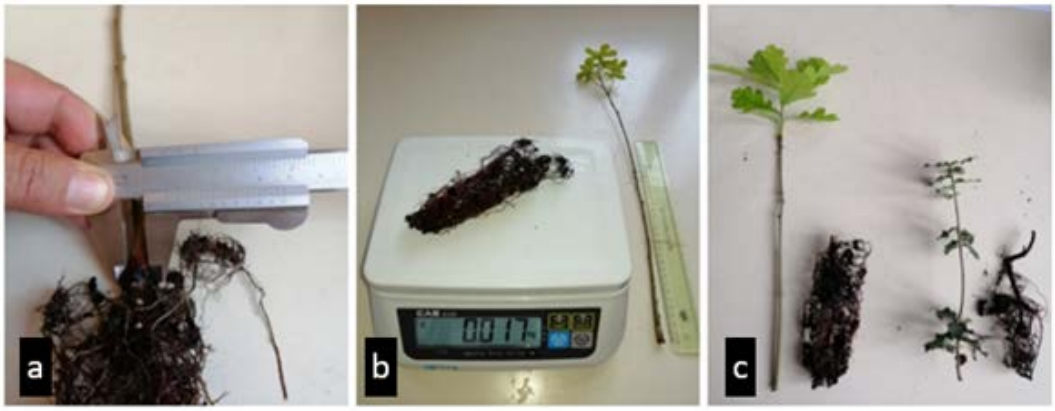


Figure 4. Determination of mycorrhizal colonization rate a) measurement of diameter b) measurement of weight and height c) preparation for mycorrhizal counting.

Mycorrhizal (Figure 5) and contaminated root pieces were examined under an Olympus SZX5 stereo microscope (Agerer, 1991; Zambonelli et al., 1993; Özderin et al., 2018). Mycorrhizal colonization rates were calculated according to the formula of Fischer and Colinas (1996):

PT= T/(N+C) (1)

PC= C/T (2)

PT: Mycorrhiza ratio

PC: Contamination rate

T: Number of mycorrhizal root pieces

N: Number of root fragments without mycorrhiza

C: Count of contaminated root parts

Figure 5. The mycorrhizal structure on the roots under the stereo microscope.

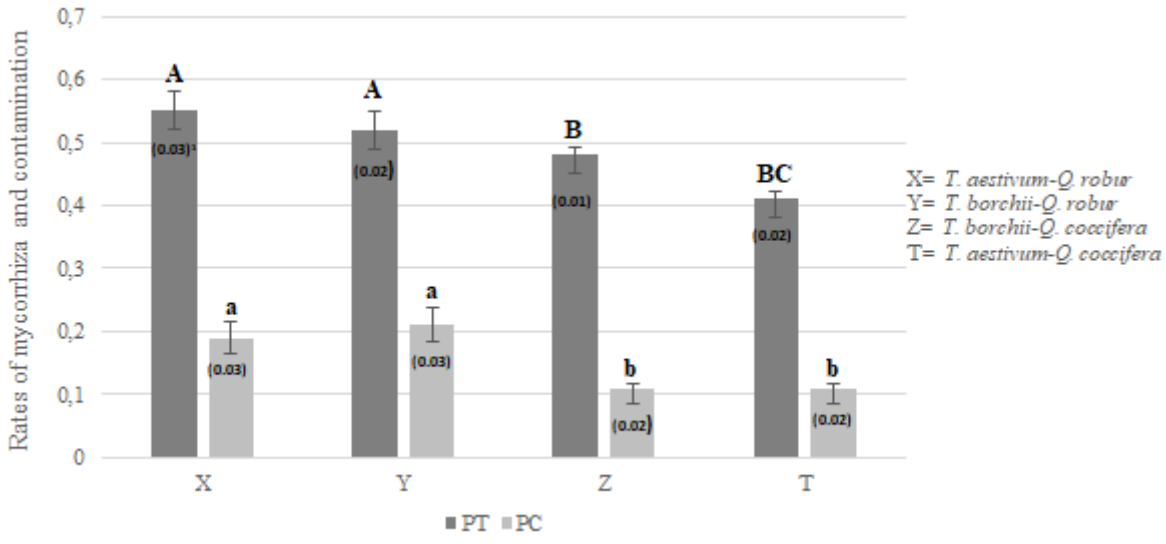
2.2.4. Statistical Analysis

Data were analysed using Minitab 16 statistics software. Firstly, an analysis of variance (ANOVA) was carried out and when significant differences were found, a Duncan test was applied to determine different groups.

3. Results

At the end of the study, both *T. borchii* and *T. aestivum* had produced well-formed ectomycorrhizae on inoculated seedlings of *Q. robur* and *Q. coccifera*. In total, 220 and 260 seeds germinated, respectively, from 300 seeds each of *Q. robur* and *Q. coccifera*. The germination percentage of *Q. robur* was 73%, whereas the germination percentage of *Q. coccifera* was 87%.

Mycorhizal colonization ratios (PT) of *Q. robur* seedlings inoculated with *T. aestivum* ranged between 0.47 and 0.68, while the PC ranged between 0.10 and 0.25, yielding a mean PT rate of 0.55 and a mean PC rate of 0.19. Similarly, the PT for *Q. robur* seedlings inoculated with *T. borchii* ranged from 0.46 to 0.71, whereas contamination rates (PC) were between 0.13 to 0.35, yielding a mean PT rate of 0.52 and a PC rate of 0.21 (Figure 5). Mycorrhizal roots were not found in the control seedlings of *Q. robur*. For *Q. coccifera* seedlings inoculated with *T. aestivum*, the PT ranged between 0.19 and 0.51, while the PC was between 0.00 and 0.25, yielding a mean PT rate of 0.41 and a PC rate of 0.11. The PT for *Q. coccifera* seedlings inoculated with *T. borchii* ranged from 0.38 to 0.56, while the PC was 0.05 to 0.18, yielding a mean PT rate of 0.48 and a PC rate of 0.11 (Figure 6). Mycorrhizal roots were not found in *Q. coccifera* seedlings under control conditions. ANOVA showed that PT rates differed significantly between the two *Quercus* species (F=0.939 and p<0.05).



[1]: Standart Error

Figure 6. Mean mycorrhiza (PT= T/(N+C) and contamination (PC= C/T) rates for Q. robur, and Q. coccifera seedlings (The columns showed with the same letter are statistically in the same homogeneous group).

Duncan test, applied to the data following the ANOVA, suggested that mycorrhiza formation rates of *Q. coccifera* and *Q. robur* seedlings for both *T. borchii* and *T. aestivum* were in the same homogeneous group (Table 1).

Table 1. Duncan test results of mycorrhiza formation rates of Q. coccifera and Q. robur seedlings.

Species	N	Group 1	Group 2
T. borchii	10		0,5300
T. aestivum	10		0,5530
Cont.	10	0,0000	
Sig.		1,000	0,403

N: Replication

There was a highly significant difference between the values of morphological characteristics of seedlings ($p < 0.001$). Measurements of morphological features showed that an average root weight of *Q. robur* seedlings inoculated with *T. aestivum* was 28.60 gr, capillary root weight 19.40 gr, stem weight 6.0 gr, stem length 42.70 cm, root collar diameter 0.66 cm. Measurements of morphological features showed that an average root weight of *Q. robur* seedlings inoculated with *T. borchii* was 28.50 gr, capillary root weight 18.60 gr, stem weight 5.30 gr, stem length 35.30 cm, root collar diameter 0.64 cm. The average root weight of *Q. robur* control seedlings was 28.00 g, capillary root weight 18.80 g, stem weight 5.60 gr, stem length 33.20 cm, root collar diameter 0.62 cm (Table 2).

Measurements of morphological features showed that an average root weight of *Q. coccifera* seedlings inoculated *T. aestivum* was 12.60 gr, capillary root weight 6.70 gr, stem weight 3.60 gr, stem length 23.70 cm, root collar diameter 0.45 cm. Measurements of morphological features showed that an average root weight of *Q. coccifera* seedlings inoculated *T. borchii* was 11.60 gr, capillary root weight 6.10 gr, stem weight 3.50 gr, stem length 20.90 cm, root collar diameter 0.46 cm. The average root weight of *Q. robur* control seedlings was 10,80 g, capillary root weight 5.80 g, stem weight 3.20 gr, stem length 20.80 cm, root collar diameter 0.44 cm (Table 2).

The different groups were identified according to Duncan’s test and are shown by different letters in each column of the Table 2.

Table 2. Morphological characteristics of seedlings inoculated by truffles.

Species of seedlings	Species of inoculums	Weight of the roots (gr)	Weight of the capil. roots (gr)	Weight of the stem (gr)	Length of the stem (cm)	Diameter of the root collar (cm)
Q. robur	*T. aestivum*	28.60 (0.9)[1] a[2]	19.40 (0.06) a	6.00 (1.2) a	42.70 (1.3) a	0.66 (0.04) a
Q. robur	*T. borchii*	28.50 (1.1) ab	18.60 (0.09) b	5.30 (0.8) c	35.30 .(0.9) b	0.64 (0.01) ab
Q. robur	Cont.	28.00 (0.7) b	18.80 (0.07) bc	5.60 (0.9) b	33.20 (1.4) c	0.62 (0.03) b
Q. coccifera	*T. aestivum*	12.60 (1.2) b	6.70 (0.05) b	3.60 (1.5) bc	23.70 (0.9) b	0.45 (0.01) ab
Q. coccifera	*T. borchii*	11.60 (0.9) ab	6.10 (0.05) ab	3.50 (1.1) b	20.90 (0.9) ab	0.46 (0.3) b
Q. coccifera	Cont.	10.80 (0.5) a	5.80 (0.03) a	3.20 (0.9) a	20.80 (1.9) a	0.44 (0.02) a

1: Standard deviation, 2: Groups by Duncan test ($p<0.001$).

4. Discussion

For over 30 years, large-scale spore inoculation programmes for *Tuber* species have been used in commercial nurseries. *Tuber melanosporum*, *T. aestivum*, and *T. borchii* are the most commonly used species used in inoculum, reflecting the commercial importance of these truffles and their ease of use in artificial inoculations.

Spore-based inoculations have numerous advantages over other methods. Inoculum is relatively cheap, easy to prepare and less time-consuming compared with mycelial inoculum produced in culture (Karwa et al., 2011; Iotti et al., 2012). This work showed that the two European truffle species, *T. borchii* and *T. aestivum*, colonized roots of both *Q. robur* and *Q. coccifera* seedlings when inoculated with standard spore-based truffle inoculation practices. The certification standards set by Fisher and Colinas (1996) suggested that *Q. robur* is the oak species producing the most successful mycorrhizal growth rate for both of these *Tuber* species. The inoculation process was successful for *Q. robur* (PT> 0.50), according to the criteria reported by Fischer and Colinas (1996) . Crucially, *T. aestivum* infection was greater on *Q. robur*, whereas *T. borchii* better infected *Q. coccifera* roots. Both *T. borchii* and *T. aestivum* spore inoculations produced well-formed ectomycorrhizae on seedlings. Contamination rates were acceptable for both seedling species and *Tuber* species (Contaminants = no more than 25% of colonized root tips).

Özderin et al. (2018) reported that the mycorrhization rate of roots with *T. aestivum* (PT) was highest in *Q. robur*, compared with *Q. coccifera* and *Q. ilex*. Similarly, *Q. robur* was the oak species with the highest *T. aestivum* development rate in our study. Differences in mycorrhizal colonization rates between these two studies may be due to the incubation period used and other incubation conditions, such as type of pot used. The bigger pots used in the

experiments described here may have provided a better opportunity for the plants to establish better roots. Also, this difference may be due to the inoculation method used.

The presence of contaminated roots in control seedlings indicates undesirable mycorrhizal contamination in the greenhouse environment or deficiencies in sterilization protocols during the mycorrhizal inoculation process. Although it is difficult to carry out sterile studies in nursery conditions, inoculation should be carried out meticulously and critical attention paid to the cleanliness of the greenhouse environment. In addition, the presence of seed-borne endophyte fungi should not be ignored. In future studies, using molecular techniques, contaminant fungi could be described and the seed and seedling mycobiome determined.

A possible reason for the higher PT values in both *T. borchii* and *T. aestivum* in *Q. robur* seedlings compared to *Q. coccifera* seedlings may be that *Q. robur* forms more capillary roots with its stronger root system. Capillary root weight of control seedlings was significantly different between the two host species. This situation may have enabled a higher PT value in *Q. robur* due to mycorrhizal formation. Changes in ectomycorrhizal development can be investigated with maintenance studies that include root pruning, variations in irrigation and nutrition programmes that increase capillary root formation in the roots of *Q. coccifera.*

In this study, for both host types of mycorrhiza inoculation, compared to control seedlings, the mycorrhizal associations had a clear, positive contribution to host morphological characteristics such as height, root collar diameter and capillary root weight. In order to make this correlation stronger, follow up-work should be carried out with measurements at different intervals. Thus, the contribution of mycorrhizal inoculation to the morphological development of seedlings can be better evaluated.

According to the seedling quality standards (Genç, 2007), *Q. robur* seedlings inoculated *T. aestivum* are first class hosts, while the control plants and *T. borchii* inoculated *Q. robur* seedlings are in the second class category. *Quercus coccifera* seedlings, on the other hand, are in the first class in terms of root collar diameter and under the 2nd class in terms of seedling height. It is known that *Q. robur* seedlings initially grow very quickly but the growth rate decreases subsequently. In contrast, *Q. coccifera* seedlings grow slowly in the beginning, but increase in the following periods (Öztürk, 2013). For this reason, individual quality standards can be used for different tree species.

Mycorrhiza formation rates for seedlings of both *Q. coccifera* and *Q. robur* seedlings with *T. borchii* and *T. aestivum* were in the same homogeneous group, based on the Duncan test (Table 2). Clearly, climate and soil characteristics should be taken into account in the

establishment of truffle orchards of both seedlings and mycorrhiza species. In addition, mycorrhizal root infection should be followed at regular intervals to determine intensity. Donnini et al. (2014) showed that mycorrhization increased with time after inoculation in inoculated seedlings, with the highest percentage of *Tuber* spp.-infected roots, as well as the highest contamination rates, were detected three years after inoculations. Zambonelli et al. (2005) showed that a 30 % initial rate of root colonization with *T. aestivum* increased to 50–70 % in mycorrhizal seedlings 5 years after planting in a suitable soil.

5. Conclusion

This work revealed that the European truffle species *T. borchii* and *T. aestivum* can colonize the roots of both *Q. robur* and *Q. coccifera* seedlings when inoculated with a standard spore-based truffle inoculation protocol. According to the certification standards set by Fisher and Colinas (1996), *Q. robur* is the oak species with the most efficient mycorrhizal growth rate for both *Tuber* species used here. There was, however, no significant difference in terms of mycorrhizal colonization between the truffle types inoculated onto the seedlings within the same plant group. The presence of inoculum had a positive effect on the growth both seedling species. In addition, differences in the development of morphological characteristics of the two *Quercus* species were found.

Truffle cultivation is a long-term process, but it is known that truffle harvesting can occur more successfully in cultivation areas when suitable conditions are provided. For this reason, it is important to plant hosts with known efficient mycorrhizal colonization in cultivation areas. To ensure a successful investment, it is critical that the seedlings used are correctly certified. As in other countries where the truffle industry is progressing, separate certification standards should be established for different host species in Türkiye. In addition, studies on truffiere management should be expanded. Thus, seedling production techniques can be developed in accordance with each host and truffle species. Faster certification processes should be carried out with the use of molecular methods by suitable institutions, in addition to the morphological and physical methods applied. Finally, university-industry cooperation should be supported for the successful mass production of truffle-inoculated seedlings.

Acknowledgments

This study was produced from the master thesis titled 'Determination of mycorrhizal colonization success in some forest tree seedlings inoculated with *Tuber aestivum* Vittad. and

Tuber borchii Vittad.'. We would like to thank Denizli Regional Directorate of Forestry and Nursery Directorate for providing all kinds of support for the realization of the study. We also would like to give very special thanks to Niyazi Uluçoban and Biologist Özge Denli for their support to our study.

References

Anonymus, (2014) Türüf ormanı eylem planı. Access address: http://trufmer.mu.edu.tr/Newfiles/330/Content/Tr%C3%BCf%20Orman%C4%B1%20Eylem%20Plan%C4%B1.pdf. Access date: 20.10.2022.

Anonymus, (2020). Türkiye Orman Varlığı. Access address: https://www.ogm.gov.tr/tr/ormanlarimizsitesi/TurkiyeOrmanVarligi/Yayinlar/2020%20T%C3%BCrkiye%20Orman%20Varl%C4%B1%C4%9F%C4%B1.pdf. Access date: 05.11.2022.

Akkemik, Ü., Sevgi, O., Yılmaz, H., Sevgi, E., ve Yılmaz, Y. (2019). Herdem Yeşil Meşelerin Türkçe Adları Üzerine Bir Değerlendirme. *Avrasya Terim Dergisi, 7*(1), 26-33.

Agerer, R. (1991). Characterization of ectomycorrhiza. In: Norris JR, Read DJ, Varma A (eds) Techniques for the study ofmycorrhiza Methods Microbiol, *23*, 25–73.

Avis, P.G., McLaughlin, D.J., Dentinger, B.C., & Reich, P.B. (2003). Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of Russula spp. in a temperate oak savanna. *New Phytol, 160,* 239–253

Berch, S. M., & Bonito, G. (2016). Mycorrhiza, Truffle diversity (*Tuber*, Tuberaceae) in British Columbia, *26*(6), 587-594.

Bonito, GM., Gryganskyi, AP., Trappe, JM., % Vilgalys, R. (2010). A global meta-analysis of Tuber ITS rDNA sequences, species diversity, host associations and long-distance dispersal.

Council Directive 1999/105/EC of 22 December (1999). On the marketing of forest reproductive material. Official Journal of the European Communities, 11:17-40.

Donnini, D., Benucci, G. M., Bencivenga, M., & Falini, L. B. (2014). Quality assessment of truffle-inoculated seedlinghs in Italy: proposing revised parameters for certification. *Forest systems, 23*(2), 385-393.

Fischer, C., & Colinas, C. (1996). Methodology for the certification of *Quercus ilex* seedlings inoculated with *Tuber melanosporum* for commercial application. First International Conference in Mycorrhizae, August 4-9, Berkeley, California, USA.

Giorgio Marozzi, G., Sánchez, S., Benucci, G.M. N., Bonito, G., Falini, L. B., Albertini, E., & Donnini, D. (2016). Mycorrhization of pecan (*Carya illinoinensis*) with black truffles: *Tuber melanosporum* and *Tuber brumale*, *Mycorrhiza, 27*(3)1-7.

Gryndler, M., Hršelová, H., Soukupová, L., Streiblová, E., Valda, S., Borovička, J., Gryndlerová, H., Gažo, J., & Miko, M. (2011) Detection of summer truffle (*Tuber aestivum* Vittad.) in ectomycorrhizae and in soil using specific primers. FEMS Microbiol Lett 318:84– 89. doi:10.1111/j.1574-6968.2011.02243.x

Karwa, A., Varma, A., & Rai, M. (2011). Edible ectomycorrhizal fungi: cultivation, conservation and challenges. In: Rai M, Varma A (eds), Diversity and biotechnology of ectomycorrhizae, Soil biology 25. Springer, Berlin, 429–453.

Kessell, S.L. (1927). Soil organisms. The dependence of certain pine species on a biological soil factor. *Empire Forestry, 6*, 70-74.

Langeron, M., & Vanbreuseghem, R. (1952). Mycology. General mycology, human and veterinary mycology. Techniques.

Leonardi, P., Murat, C., Puliga, F., Iotti, M., & Zambonelli, A. (2020). Ascoma genotyping and mating type analyses of mycorrhizas and soil mycelia of *Tuber borchii* in a truffle orchard established by mycelial inoculated plants. *Environmental microbiology, 22*(3), 964-975.

Iotti, M., Piattoni, F., & Zambonelli, A. (2012). Techniques for host plant inoculation with truffles and other edible ectomycorrhizal mushrooms. In Edible ectomycorrhizal mushrooms, 145-161, Springer, Berlin, Heidelberg.

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

Mukerji, K.G., Raina, S., & Chamola, B.P. (2000). Evolution of mycorrhiza, Mycorrhizal Biology, Kluwer Academic Publishers, New York, Editors: Mukerji K.G., Chamola B.P., Singh J., 1–25.

Ortaş, İ. (1998). Toprak ve Bitkide Mikoriza. Workshop, 61,Çukurove Üniversitesi Ziraat Fakültesi Toprak Bölümü, 20-22 Mayıs, Adana.

Özderin, S., Yılmaz, F., & Alli, H. (2018). Determining mycorrhiza rate in some oak species inoculated with *Tuber aestivum* Vittad. (summer truffle). *Turkish Journal of Forestry, 19*(3), 226-232.

Öztürk, S. (2013). Türkiye'nin Meşeleri Teşhis ve Tanı Kılavuzu. Orman Genel Müdürlüğü, Orman Zararlılarıyla Mücadele Dairesi Başkanlığı.

Reyna, S., Boronat, T., & Palomar, E. (2000). Control decalidad en la planta micorrizada con *Tuber melanosporum* Vitt. producida por viveros comerciales. *Montes, 61*, 17–24

Smith, S. E., & Read, D. J. (2008). Mycorrhizal Symbiosis, Academic Press, San Diego, USA

Türkoğlu, A. (2015). Yeraltındaki Gizli Hazine: Trüf Mantarı. T.C. Orman Ve Su İşleri Bakanlığı Orman Genel Müdürlüğü.

Yuanzhi, T. (2016). Method for cultivating wild truffles -Google Patents,(CN105349435A)

Zambonelli, A., Gıunchedı, L., & Pollini, C. P. (1993). An enzyme-linked immunosorbent assay for the detection of *Tuber albidum* ectomycorrhiza. Symbiosis.

Zambonelli, A., Iotti, M., Giomaro, G., Hall, I., & Stocchi, V. (2002). *T. borchii* cultivation: an interesting perspective. Edible mycorrhizal mushrooms and their cultivation. In: Hall I, Yun W, Danell E, Zambonelli A (eds) Proceedings of the Second International Conference on Edible Mycorrhizal Mushrooms, 3–6 July 2001. Christchurch, New Zealand (CD-Rom).

Zambonelli, A., Iotti, M., Zinoni, F., Dallavalle, E., & Hall, I. R. (2005). Effect of mulching on Tuber uncinatum ectomycorrhizas in an experimental truffière. *New Zealand Journal of Crop and Horticultural Science, 33*(1), 65-73.