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ORIGINAL PAPER

Colonization of selected host plant species by Burgundy truffle under greenhouse conditions

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ABSTRACT

Burgundy truffle *Tuber aestivum* Vittad. syn. *T. uncinatum* Chatin can be a profitable agroforestry crop outside the Mediterranean region. Truffle fungi grow symbiotically as ectomycorrhizae on the roots of host trees, notably hazels and oaks. In some parts of Poland, conditions appear favourable for cultivation of Burgundy truffle, but how management practices affect truffle establishment and fruiting remains to be studied. In a greenhouse study, we tested two inoculation techniques that differed in the method and timing of inoculum application on mycorrhizal colonization of *Pinus sylvestris* L., *Larix decidua* Mill., *Quercus robur* L., and *Fagus sylvatica* L. We found that the method of *T. aestivum* spore application under greenhouse conditions had no significant effect on mycorrhization efficiency, regardless of host plant species. Mycorrhization of larch, pine, beech, and oak seedlings by *T. aestivum* did not differ in time to infection or degree of mycorrhizal root infection. However, oak seedlings had a higher degree of mycorrhization, which explains the popularity of this species in truffle plantations.

KEY WORDS

Agroforestry, coniferous tree species, deciduous tree species, ectomycorrhiza, *Tuber aestivum*

Introduction

Since the mid-1990s, European policy has promoted land management systems that combine production, environmental services (biodiversity, carbon sequestration, nutrient cycling, and water quality), and social benefits, which has renewed interest in agroforestry systems (Mosquera--Losada *et al.*, 2012; Thomas *et al.*, 2022). Agroforestry combines the use of land for crop production (*e.g.*, medicinal plants and plants for bees, edible mushrooms) or for grazing animals is combined with forest cover. This approach to land use may be more beneficial than conventional agriculture or forestry in some regions (Dzierżyńska, 2011; Hilszczańska, 2015). Truffles are becoming more popular as an agroforestry crop since they can be grown in tree plantations that provide integrated land use of marginal lands (Bonet *et al.*, 2006; Reyna-Domenech and García-Barreda, 2009; Benucci *et al.*, 2012).

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In addition to their economic and social importance, truffle plantations also contribute to nature conservation (Samils, 2002; Samils *et al.*, 2008). For example, plantations of *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. support a large number of moss, lichen, fungus, and invertebrate species (Gärdenfors, 1994). Tree and shrub species diversity in truffle plantations increases the biodiversity of field and forest ecosystems. A forest rich in tree species provides niches for numerous invertebrate species, including pollinating insects, as well as for small vertebrates (mice, moles, shrews, lizards, squirrels) and nesting sites for birds (Urban, 2016). They also contribute to the attractiveness of the landscape by forming field copses. Since the establishment of the first truffle plantations (truffle orchards) in France, Italy, and other parts of the world, considerable research has been done to improve their productivity (Sourzat *et al.*, 1993; Zambonelli *et al.*, 2005; Garcia-Montero *et al.*, 2012). The key factor affecting truffle fructification in plantations is the persistence of truffle mycorrhizae on the roots of host plants over many years (De Miguel *et al.*, 2014).

The production of tree seedlings with mycorrhiza by various truffle species under laboratory or greenhouse conditions began in the early 1970s in France and Italy. The first successful truffle harvests from artificially inoculated mycorrhizal trees were reported in France in the late 1970s (Chevalier and Frochot, 1997). Truffle seedling inoculation methods developed in Europe half a century ago are now universally applicable and provide good results for Asian and American host plant species and compatible truffle species (Kinoshita *et al.*, 2018; Freiberg *et al.*, 2021). Currently, the most commonly used truffle species for commercial seedling inoculation are black truffle *Tuber melanosporum* Vittad. and Burgundy truffle (syn. summer truffle) *Tuber aestivum* Vittad. Interest in commercial truffle production is high as yields in natural sites decline and market demand increases (Shamekh *et al.*, 2014).

The maintenance of mycorrhizal truffles in plantations is influenced by many factors, including: age of plantation, soil factors such as pH, calcium carbonate, and nutrients content (García-Montero *et al.*, 2009; Suz *et al.*, 2010, Chevalier and Sourzat, 2012; Thomas 2012); soil moisture (Olivera *et al.*, 2011; Pacioni *et al.*, 2014); climatic conditions (Wedén *et al.*, 2004; Ponce *et al.*, 2010); management practices; and host plant species (De Miguel *et al.*, 2014). A truffle orchard with multiple host plant species may provide additional benefits, although there are few studies on this subject. Orchards with multiple host plant species, as well as other plant species that support truffle mycorrhizal development and positively influence soil conditions, are characterized by higher and more durable truffle fruiting bodies. Research at sites where truffles occur naturally shows that both host plant species richness of host plants and other non-host forest floor plants are beneficial for truffle yield (Hilszczańska *et al.*, 2019a). In the case of fast-growing host plant species (*e.g.*, hazel, birch), fruiting bodies may appear as early as six years after orchard establishment, while the presence of slower growing tree and shrub species extends plantation longevity.

A greater diversity of tree and shrub species increases the stability of the orchard as well as its resistance to unfavourable weather conditions, fungal diseases, insect infestations, etc. Truffle orchards with only one host species are more vulnerable to extremes such as drought, hail, and other severe weather caused by dynamic climate warming (Thomas and Büntgen, 2019). For example, climate warming may cause beech *Fagus sylvatica* L. forests to be replaced by oak *Quercus* spp. forests (Leuschner, 2020; Kasper *et al.*, 2021). Furthermore, in some climate change scenarios, rising global temperatures favour the spread of Burgundy truffle in Europe. The results of Čejka *et al.* (2020) and Thomas and Büntgen (2017) suggest that Burgundy and Périgord truffles could become an important and high-value crops in many European regions where suitable calcareous soils exist. However, current truffle production areas are expected to decline (Čejka *et al.*, 2022)

The aim of this research was to determine which host plants achieve the highest mycorrhization rate when inoculated with Burgundy truffle *T. aestivum*. In addition, we investigated the efficacy of two inoculation methods under greenhouse conditions that differed in the method and timing of *T. aestivum* spores application to the substrate. We hypothesized that roots of deciduous trees would be more effectively colonized by Burgundy truffle (in terms of numbers and mycorrhization rates) than those of conifers, and that simultaneous application of the inoculum with sowing of host plant seeds would result in higher mycorrhization rates.

Material and methods

PREPARATION OF THE INOCULUM. The fruiting bodies of *T. aestivum* were collected from selected sites in the Nida Basin (southern Poland), where the soils are conducive to this fungus. After harvesting, the fruiting bodies were briefly stored in sterile moist sand at 4°C. This preservation method maintains the infectivity of the fungus (colonisation potential) for up to 2 years (Zambonelli *et al.*, 2010) and improves spore germination, possibly due to the activity of microorganisms present in the fruiting bodies (Hall *et al.*, 2007).

The collected fruiting bodies were cleaned and samples were taken from the *gleba* to assess their maturity. Samples were examined under an Axioskop 2 plus optical microscope at 10× to 100× magnification. Maturity of fruiting bodies was assessed by the degree of spore development according to a 5-point scale (Büntgen *et al.*, 2017). Fully mature fruiting bodies (grade 5) were selected for inoculum preparation. The selected fruiting bodies were first dried at 40°C for 24 hours and then ground and stored in this form in the refrigerator at 4°C.

GROWING AND INOCULATION OF TREE SEEDLINGS. Seeds of *Pinus sylvestris* L., *Larix decidua* Mill., *Q. robur* and *F. sylvatica* were obtained from stands in the Chrzanów and Kłobuck forest districts in southern Poland. Seeds were sown on May 5, 2021, on vermiculite-peat (4:1, v:v) growing medium (pH=6.5). Calcium carbonate ($CaCO_3$) was added at a rate of 48 g per litre of medium. The growing medium was sterilized at 121°C for 1 hour prior to sowing seeds. The soil pH at sowing was 7.2. The growing medium was placed in 1-L pots. For each tree species, 70 pots (replicates) were prepared.

Two methods of inoculation were tested. In the first method, 30 replicates (pots) of each host plant species were inoculated by adding 1.5 g of dry inoculum per container and mixing it evenly into the soil before sowing. In the second inoculation method, 1 g of ground spores in 25 ml of distilled water was added to each of the 30 pots four weeks after sowing, when the fine roots of the host plants had formed. A control treatment with 10 pots of each host plant species was carried out without the addition of inoculum.

Seedlings were grown in a greenhouse at constant temperature for 11 months. Humidity and moisture content of the substrate were monitored, and the pots were watered as needed, usually twice a week. After 3, 6, and 11 months of growth, fine roots were taken from selected seedlings to check for mycorrhization.

Mycorrhization of each seedling was visually determined by the morphological characteristics of the living ectomycorrhizal roots, which were rated using a 4-point scale (percentage of roots tips with *T. aestivum* mycorrhizae):

– 0-25% – grade 1,
– 26-50% – grade 2,

– 51-75% – grade 3,
– 76-100% – grade 4.

To confirm mycorrhizae species, root tips of seedlings (approximately 100 fine roots per seedlings), were taken randomly and their features were observed using a Zeiss Stemi 2000 C stereoscopic microscope (magnification from 10 to 80×). Mycorrhizae were identified based on morphology using published keys and descriptions (Agerer, 1987-2006; Ingleby *et al.*, 1990; Zambonelli *et al.*, 1993; Agerer and Rambold, 2004-2007) as well as the DEEMY portal (http://www.deemy.de/) and the Forest Research Institute database (Hilszczańska, 2016).

Statistical analysis

Statistical analyses were carried out using Statistica 13.1 (TIBCO Software Inc., 2017). To assess the significance of differences in mycorrhization among tree species and in relation to the inoculation method used, a Kruskal-Wallis one-way analysis of variance by ranks test was performed ($p<0.05$). Multiple comparison tests were performed to distinguish homogeneous groups (multiple comparison test of mean rank for all samples). This procedure was used due to unequal sample sizes in the groups and the lack of a normal distribution (as demonstrated by the Shapiro-Wilk test).

Results

Mycorrhizal infections with *T. aestivum* were identified on all host plant species, whereas no roots were infected with *T. aestivum* in the control treatment, although some roots of control seedlings were infected with mycorrhizae of *Thelephora terrestris* Ehrh. and *Tomentella* sp. In coniferous species, mycorrhization by *T. aestivum* was lower than in deciduous species, but the differences were not significant (Fig. 1). Differences in the degree of mycorrhization were significant only when inoculated treatments were compared with the control treatment (without inoculation). The average degree of mycorrhization, assessed according to the four-point scale for both coniferous and deciduous species, was 2 (26-56% mycorrhiza).

Irrespective of inoculation method, the degree of mycorrhization was significantly higher in oak than in pine and beech ($p=0.001$, Fig. 2). The degree of mycorrhization of oak and larch was not significantly different. The mycorrhizal infection of larch, pine and beech was also not significantly different.

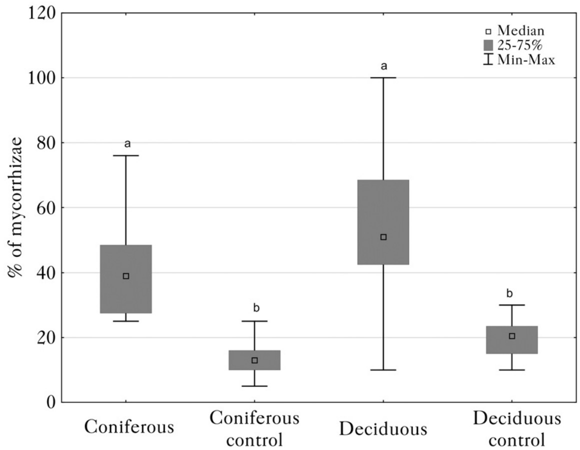

Fig. 1.

Percentage of roots with Tuber aestivum mycorrhizae on inoculated coniferous and deciduous trees and of non-target mycorrhizae in control treatments. Treatments with the same letter did not differ significantly in a multiple comparison test of mean rank for all samples at $p<0.05$.

The inoculation method did not affect the infection rate by *T. aestivum* mycorrhizae in any species (Figs. 3-6), so inoculation at the time of sowing (method 1) and inoculation four weeks after sowing by introducing spores into the growing medium (method 2) made no difference (Table 1). The average degree of mycorrhization was 2 (26-50% mycorrhizae) for pine, larch, and beech and 3 (51-75%) for oak regardless of the method of mycorrhizal inoculation.

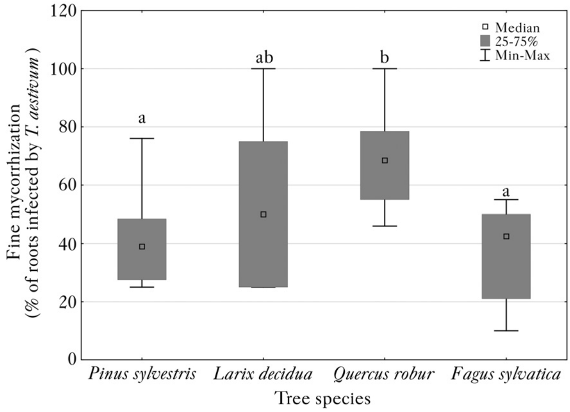

Fig. 2.

Percentage of root tips with Tuber aestivum mycorrhiza on *Pinus sylvestris*, *Larix decidua*, *Quercus robur*, and *Fagus sylvatica*. Species with the same letter did not differ significantly based on a mean rank multiple comparison test of all samples at $p<0.05$.

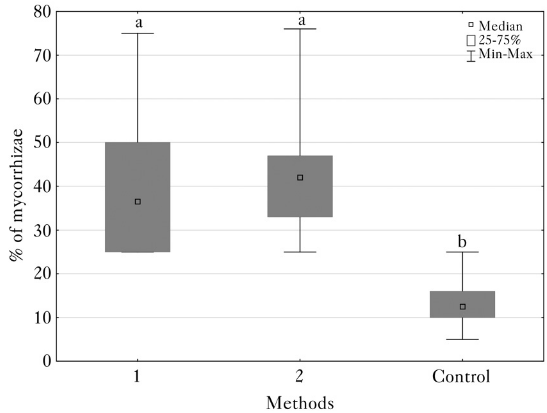

Fig. 3.

Percentage of roots with *Tuber aestivum* mycorrhiza on *Pinus sylvestris* seedlings for two inoculation methods. Only non-target mycorrhizae species were present in the control seedlings. Treatments with the same letter did not differ significantly in a mean-rank multiple comparison test for all samples at $p<0.05$.

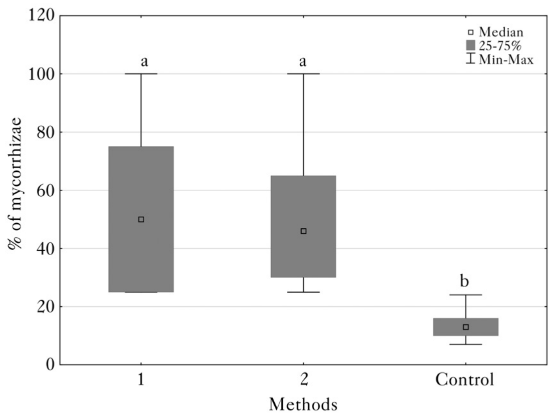

Fig. 4.

Percentage of roots with *Tuber aestivum* mycorrhiza on *Larix decidua* seedlings for two inoculation methods. Only non-target mycorrhizae species were present in control seedlings. Treatments with the same letter did not differ significantly based on a mean-rank multiple comparison test of all samples at $p<0.05$.

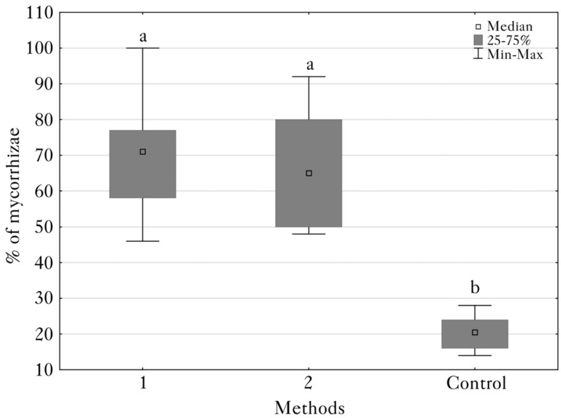

Fig. 5.
Percentage of roots with *Tuber aestivum* mycorrhiza on *Quercus robur* seedlings for two inoculation methods. Only non-target mycorrhizae species were present in control seedlings. Treatments with the same letter did not differ significantly based on a mean-rank multiple comparison test of all samples at $p<0.05$.

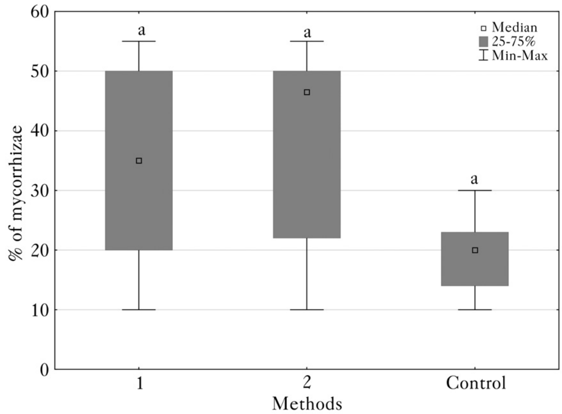

Fig. 6.
Percentage of roots with *Tuber aestivum* mycorrhiza on *Fagus sylvatica* seedlings in two inoculation methods. Only non-target mycorrhizae species were present in the control seedlings. Treatments with the same letter did not differ significantly based on a mean-rank multiple comparison test for all samples at $p<0.05$.

Table 1.
Percentage of roots with mycorrhiza on seedlings inoculated by two methods (median values and the results of the mean-rank multiple comparison test, with significant differences within species indicated by an asterisk)

Tree species	Mycorrhizal roots [%] inoculation method			
	1	2	control	*p*-value
Pinus sylvestris	36.5	42.0	12.5*	0.001
Larix decidua	50.0	46.0	13.0*	0.001
Quercus robur	71.0	65.0	20.5*	0.001
Fagus sylvatica	35.0	46.5	20.0	0.060

The degree of mycorrhization by non-target mycorrhizae was at level 1 (0-25%) for pine, oak, and larch, well below the degree of mycorrhization by *T. aestivum* in these species. In contrast, mycorrhization by non-target mycorrhizae in beech was at level 2 (26-50%), which was not significantly different from the degree of mycorrhization by *T. aestivum* in this species.

Discussion

Fungi of the genus *Tuber* (truffle) form mycorrhiza with a large number of plant species. Burgundy truffle (*T. aestivum*) forms a mycorrhizal symbiosis with over 20 species of trees and shrubs from the genera *Abies*, *Betula*, *Carpinus*, *Carya*, *Castanea*, *Cistus*, *Corylus*, *Fagus*, *Ostrya*, *Tilia*, *Picea*, *Pinus*, *Populus*, *Quercus*, *Larix* and *Ulmus* (Wedén *et al*., 2009; Benucci *et al*., 2012; Stöbbe *et al*., 2013a, b; Gryndler *et al*., 2014). The most common host plants for Burgundy truffle in Poland are oak, hornbeam, linden, and hazel (Hilszczańska *et al*., 2008).

In this trial, the compatibility of *T. aestivum* with two coniferous species, Scots pine and European larch, was tested to determine their potential use to increase host species diversity in truffle plantations. Infection of juvenile seedlings was evaluated to determine mycorrhiza development after 9 months of seedling growth. Mycorrhizae of Burgundy truffle with English oak (Fig. 4) and common beech showed degrees of infection similar to Scots pine and European larch. However, oak had a greater average number of mycorrhizal roots than the other species. The hypothesis that roots of deciduous trees are more effectively colonized (in terms of number and time) by Burgundy truffle than those of conifers holds true in the case of oak.

Competition from undesirable mycorrhizae species may hinder infection by *T. aestivum*. Non-target mycorrhizae are most often formed by fungi belonging to the genus *Thelephora* and *Scleroderma* (Hall *et al*., 2007). In our experiment, mycorrhizal infection by *Th. terrestris* and *Tomentella* sp. occurred only in the control treatment, which may indicate that Burgundy truffle outcompeted other mycorrhizal fungal species. The high level of colonization by Burgundy truffle found in this trial may have resulted from the method used to preserve the fruiting bodies prior to inoculation.

To obtain mycorrhizal seedlings, several types of natural and laboratory-produced mycorrhizal inoculum are reportedly used under sterile, semi-sterile, or non-sterile conditions (Repac, 2011). Three types of inoculum are widely used: spores (gamic inoculum), pure mycelial cultures (vegetative inoculum), and colonized roots (symbiotic inoculum). An interesting aspect of the present experiment is that mycorrhizal colonization occurred regardless of the method and timing of inoculum application. This trial confirmed the effectiveness of spores as a form of inoculum. This method is widely used under greenhouse (semi-sterile) conditions (Bedini *et al*., 1999) and is recommended for fungal species with the potential to form spores in large numbers and that can colonize the host plant root system quickly and efficiently (Lu *et al*., 1998). For over 30 years, spores of *T. melanosporum*, *T. aestivum,* and *T. borchii* Vittad. have been the preferred inoculum for the mycorrhization of plants in truffle plantations (Hall *et al*., 2007; Karwa *et al*., 2011).

Some researchers (Giomaro *et al*., 2005; Zambonelli *et al*., 2008) emphasize the advantages of vegetative mycelium inoculum, which carries a lower risk of introducing undesirable fungal species and possesses a higher colonization capacity than spore inoculum. For example, some strains of *T. borchii* mycelium can form mature mycorrhizae on *Q. robur* in approximately 1 month under semi-sterile conditions (Iotti *et al*., 2016). Moreover, vegetative inoculum enables the infection of plants with fungal genotypes that have high affinity for the host plant, perform well under specific ecological conditions, and have favourable fruiting characteristics.

In comparison, spore-based inoculum is easy and cheap to prepare and can be produced quickly without the need for specialized equipment or advanced training of personnel. The spores are obtained by crushing the fruiting bodies using a mortar and pestle or an electric grinder or blender. However, considering the long lead time for mycorrhizal seedlings production, a vegetative inoculum method should be considered in the future, given the cost of vegetative mycelium production vs cultivation time of mycelium.

Of the four tree species studied in this trial, oak seedlings had the highest degree of mycorrhizal infection, which helps to explain the popularity of this species in truffle plantations, especially where Burgundy truffle is used (Wedén *et al.*, 2009; Hilszczańska, 2016). Seedlings inoculated with *T. aestivum* are the second most commonly produced planting material (after seedlings inoculated with *T. melanosporum*) used in truffle plantations (Murat, 2015). In France, the highest Burgundy truffle yields are obtained by planting species such as hazel *Corylus avellana* L. and *C. colurna* L., hornbeam *Carpinus betulus* L. and *Ostrya carpinifolia* Scop., oaks *Q. robur*, *Q. petraea*, *Q. pubescens* Wild. and *Q. cerris* L., pine *Pinus nigra* var. *austriaca* (Hoess) Badoux and cedar *Cedrus atlantica* (Endl.) G. Manetti ex Carrière (Chevalier and Frochot, 1997). In Poland, Burgundy truffle fruiting bodies were most abundant in mixed stands, where, apart from oak and hazel, there were also beeches, hornbeams, lindens, pines, and larches (Hilszczańska *et al.*, 2019a). Similar Burgundy truffle host tree species are found in Slovakia (Gazo *et al.*, 2005).

Granetti *et al.* (2005) mentioned seven pine species that serve as hosts for *T. aestivum*: *P. excelsa* Wall. ex Lamb, *P. halepensis* Mill., *P. nigra* Arn., *P. pinaster* Aiton, *P. pinea* L., *P. strobus* L. and *P. sylvestris*. Where *T. aestivum* occurs in the Nida Basin, the fruiting bodies of this species were often found in the vicinity of Scots pine (Hilszczańska *et al.*, 2019a). Hence, in this study, Scots pine was evaluated as a host species. The evaluation of European larch as a potential Burgundy truffle host was based on observations of truffle mycorrhizae on one-year- and three-year-old bare-root seedlings (Leski *et al.*, 2008). Based on DNA sequences obtained by isolation from mycorrhizae, Leski *et al.* (2008) found similarity to *Tuber oligospermum* (Tul. & C. Tul.) Trappe and *T. borchii*, although at relatively low levels of 91% and 93%, respectively.

It appears that there is a wide variety of suitable Burgundy truffle host plant species, with substrate pH being the main factor limiting mycorrhizal symbiosis with *Tuber* spp. A good example is the presence of numerous mycorrhizae and fruiting bodies of Burgundy truffle in a spruce *Picea abies* (L.) H. Karst. stand (Stöbbe *et al.*, 2013b).

The methods of mycorrhizal inoculation and rearing of infected seedlings used in Mediterranean countries worked well in the environmental conditions found in Poland. The fructification of Burgundy truffle in one of the Polish plantations (established in 2016) opens new opportunities to promote the active protection of the valued species of fungi.

Conclusions

- The rate of mycorrhization of larch, pine, beech, and oak seedlings by *T. aestivum* after 9 months since inoculation was at similar level.
- Oak seedlings tended to have a higher degree of infection by *T. aestivum* mycorrhizae than other host species evaluated, hence the popularity of this tree species in truffle plantations.
- Inoculation method did not significantly affect mycorrhization rate.

Authors' contributions

All authors substantially conceived the ideas, contributed to conceptualization, resources, writing the original draft, and reviewing and editing the text.

Conflicts of interest

Authors declare no personal circumstances or interests that may be perceived as inappropriately influencing the representation or interpretation of reported research results.

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References

Agerer, R., 1987-2006. Colour Atlas of Ectomycorrhizae. Schwäbisch-Gmünd, München: Einhorn-Verlag Eduard Dietenberger GmbH.

Agerer, R., Rambold, G., 2004-2007. DEEMY-An information system for characterization and determination of ectomycorrhizae. Munich: Ludwig Maximilians University. Available from http://www.deemy.de [accessed: 01.04.2022].

Bedini, S., Bagnoli, G., Sbrana, C., Leporini, C., Tola, E., Dunne, C., D'Andrea, F., O'Gara, F., Nuti, M.P., 1999. *Pseudomonas* isolated from within fruit bodies of *Tuber borchii* are capable of producing biological control or phytostimulatory compounds in pure culture. *Symbiosis*, 26: 223-236. Available from https://dalspace.library.dal.ca/bitstream/handle/10222/77636/VOLUME%2026-NUMBER%203-1999-PAGE%20223.pdf?sequence=1 [accessed: 25.06.2022].

Benucci, G.M.N., Bonito, G., Falini, L.B., Bencivenga, M., Donnini, D., 2012. Truffles, timber, food, and fuel: sustainable approaches for multi-cropping truffles and economically important plants. In: A. Zambonelli, G.M. Bonito, eds. *Edible ectomycorrhizal mushrooms*. Berlin, Heidelberg: Springer, pp. 265-280. DOI: http://dx.doi.org/10.1007/978-3-642-33823-6_15.

Bonet, J.A., Fischer, C., Colinas, C., 2006. Cultivation of black truffle to promote reforestation and land-use stability. *Agronomy for Sustainable Development*, 26 (1): 69-76. DOI: https://doi.org/10.1051/agro:2005059.

Büntgen, U., Bagi, I., Fekete, O., Molinier, V., Peter, M., Splivallo, R., Vahdatzadeh M., Richard, F., Murat, C., Tegel, W., Stobbe, U., Martínez-Peńa F., Sproll, L., Hülsmann, L., Nievergelt, D., Meier, B., Egli, S., 2017. New insight into the complex relationship between weight and maturity of Burgundy truffles (*Tuber aestivum*). *PLOS ONE* 12 (1): e0170375. DOI: https://doi.org/10.1371/journal.pone.0170375.

Čejka, T., Isaac, E.L., Oliach, D., Martínez-Peńa, F., Egli, S., Thomas, P., Trnka, M., Büntgen, U., 2022. Risk and reward of the global truffle sector under predicted climate change. *Environmental Research Letters*, 17 (2): 024001. DOI: https://doi.org/10.1088/1748-9326/ac47c4.

Čejka, T., Trnka, M., Krusic, P.J., Stöbbe, U., Oliach, D., Vaclaik, T., Tegel, W., Büntgen, U., 2020. Predicted climate change will increase the truffle cultivation potential in central Europe. *Scientific Reports*, 10: 21281. DOI: https://doi.org/10.1038/s41598-020-76177-0.

Chevalier, G., Frochot, H., 1997. La truffe de Bourgogne. Levallois-Perret: Editions Pétrarque, 279 pp.

Chevalier, G., Sourzat, P., 2012. Soils and Techniques for Cultivating *Tuber melanosporum* and *Tuber aestivum* in Europe. In: A. Zambonelli, G.M. Bonito, eds. *Edible ectomycorrhizal mushrooms*. Berlin, Heidelberg: Springer, pp: 163-189. DOI: https://doi.org/10.1007/978-3-642-33823-6_10.

De Miguel, A.M., Águeda, B., Sánchez, S., Parladé, J., 2014. Ectomycorrhizal fungus diversity and community structure with natural and cultivated truffle hosts: applying lessons learned to future truffle culture. *Mycorrhiza*, 24 (1): 5-18. DOI: https://doi.org/10.1007/s00572-013-0554-3.

Dzierżyńska, A., 2011. Agroleśnictwo w Europie – zacofanie czy postęp? *Postępy Nauk Rolniczych*, 63 (4): 129-141. Available from http://psjd.icm.edu.pl/psjd/element/bwmeta1.element.oai-journals-pan-pl-93102/c/oai-journals-pan-pl-93102_full-text_3db6449b-82c0-49fa-9c30-156d3a78a7be.pdf [accessed: 30.06.2022].

Freiberg, J.A., Sulzbacher, M.A., Grebenc, T., Santana, N.A., Schardong, I.S., Marozzi, G., Fronza, D., Giachini, A.J., Donnini, D., Jacques, R.J.S., Antoniolli, Z.I., 2021. Mycorrhization of pecans with European truffles (*Tuber* spp., Tuberaceae) under southern subtropical conditions. *Applied Soil Ecology*, 168: 104108. DOI: https://doi.org/10.1016/j.apsoil.2021.104108.

Gärdenfors, U., 1994. Eken-Utnyttjad av Tusentals Organismer. In: U. Olsson, ed., *Ekfrämjandet 50 år. Ekfrämjandet och Skogsvårdsstyrelsen*, Ronneby, Sweden, pp. 77-82.

García-Montero, L.G., Quintana, A., Valverde-Asenjo, I., Díaz, P., 2009. Calcareous amendments in truffle culture: A soil nutrition hypothesis. *Soil Biology and Biochemistry*, 41 (6): 1227-1232. DOI: https://doi.org/10.1016/j.soilbio.2009.03.003.

García-Montero, L.G., Valverde-Asenjo, I., Moreno, D., Díaz, P., Hernando, I., Menta, C., Tarasconi, K., 2012. Influence of edaphic factors on edible ectomycorrhizal mushrooms: new hypotheses on soil nutrition and C sinks associated to ectomycorrhizae and soil fauna using the *Tuber* brűlé model. In: A. Zambonelli, G.M. Bonito, eds. *Edible ectomycorrhizal mushrooms, current knowledge and future prospects*. Soil Biology 34. Berlin: Springer-Verlag, pp. 83-104. DOI: https://doi.org/10.1007/978-3-642-33823-6_6.

Gazo, J., Miko, M., Chevalier, G., 2005. First results of inventory research on economically important species of truffles (*Tuber*) in the Tribec Mountains. *Acta Fytotechnica et Zootechnica*, 8 (3): 66-71.

Giomaro, G., Sisti, D., Zambonelli, A., 2005. Cultivation of edible ectomycorrhizal fungi by in vitro mycorrhizal synthesis. In: S. Declerck, J.A. Fortin, D.G. Strullu, eds. *In vitro culture of mycorrhizas*. Soil biology series. Berlin: Springer, pp: 253-267. DOI: https://doi.org/10.1007/3-540-27331-X_14.

Granetti, B., De Angelis, A., Materozzi, G., 2005. Umbria, terra di tartufi. Terni: Regione Umbria, 304 pp.

Gryndler, M., Černá, L., Bukovská, P., Hršelová, H., Jansa, J., 2014. *Tuber aestivum* association with non-host roots. *Mycorrhiza*, 24: 603-610. DOI: https://doi.org/10.1007/s00572-014-0580-9.

Hall, I.R., Brown, G.T., Zambonelli, A., 2007. Taming the truffle: the history, lore, and science of the ultimate mushroom. Portland: Timber, 279 pp.

Hilszczańska, D., Sierota, Z., Palenzona, M., 2008. New *Tuber* species found in Poland. *Mycorrhiza*, 18: 223-226. DOI: https://doi.org/10.1007/s00572-008-0175-4.

Hilszczańska, D., 2015. Popularyzacja upraw truflowych w Polsce jako metody ochrony gatunkowej trufli letniej i zagospodarowania terenów nieleśnych (Popularization of truffle cultivation in Poland as a method protect *Tuber aestivum* and management non-forest areas). *Studia i Materiały Centrum Edukacji Przyrodniczo-Leśnej*, 17 (3): 119-129. Available from https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-ba039010-2b9c-4d09-b55e-d64ef257fdca [accessed: 30.06.2022].

Hilszczańska, D., 2016. Polskie trufle-skarb odzyskany: o hodowli i kulinariach podziemnego przysmaku. Warszawa: Centrum Informacyjne Lasów Państwowych, 56 pp.

Hilszczańska, D., Rosa-Gruszecka, A., Gawryś, R., Horak, J., 2019a. The effect of soil properties and vegetation characteristic in determining the frequency of fruiting bodies of the Burgundy truffle in Southern Poland. *Ecoscience*, 26 (2): 113-122. DOI: https://doi.org/10.1080/11956860.2018.1530327.

Hilszczańska, D., Szmidla, H., Sikora, K., Rosa-Gruszecka, A., 2019b. Soil properties conducive to the formation of *Tuber aestivum* Vitt. fruiting bodies. *Polish Journal of Environmental Studies*, 28 (3): 1713-1718. DOI: https://doi.org/10.15244/pjoes/89588.

Iotti, M., Piattoni, F., Leonardi, P., Hall, I. R., Zambonelli, A., 2016. First evidence for truffle production from plants inoculated with mycelial pure cultures. *Mycorrhiza*, 26: 793-798. DOI: https://doi.org/10.1007/s00572-016-0703-6.

Ingleby, K., Mason, P.A., Last, F.T., Fleming, L.V.V., 1990. Identification of ectomycorrhizas. London: HMSO, 304 pp.

Karwa, A., Varma, A., Rai, M., 2011. Edible ectomycorrhizal fungi: cultivation, conservation and challenges. In: M. Rai, A. Varma, eds. *Diversity and biotechnology of ectomycorrhizae*. Soil Biology 25. Berlin: Springer, pp. 429-453. DOI: https://doi.org/10.1007/978-3-642-15196-5_19.

Kinoshita, A., Obase, K., Yamanaka, T., 2018. Ectomycorrhizae formed by three Japanese truffle species (*Tuber japonicum*, *T. longispinosum*, and *T. himalayense*) on indigenous oak and pine species. *Mycorrhiza*, 28: 679-690. DOI: https://doi.org/10.1007/s00572-018-0860-x.

Leski, T., Rudawska, M., Aučina, A., 2008. The ectomycorrhizal status of European larch (*Larix decidua* Mill.) seedlings from bare-root forest nurseries. *Forest Ecology and Management*, 256 (12): 2136-2144. DOI: https://doi.org/10.1016/j.foreco.2008.08.004.

Leuschner, C., 2020. Drought response of European beech (*Fagus sylvatica* L.) – A review. *Perspectives in Plant Ecology, Evolution and Systematics*, 47: 125576. DOI: https://doi.org/10.1016/j.ppees.2020.125576.

Lu, X., Malajczuk, N., Dell, B., 1998. Mycorrhiza formation and growth of *Eucalyptus globulus* seedlings inoculated with spores of various ectomycorrhizal fungi. *Mycorrhiza*, 8: 81-86. DOI: https://doi.org/10.1007/s005720050216.

Murat, C., 2015. Forty years of inoculating seedlings with truffle fungi: past and future perspectives. *Mycorrhiza* 25: 77-81. DOI: https://doi.org/10.1007/s00572-014-0593-4.

Mosquera-Losada, M.R., Moreno, G., Pardini, A., McAdam, J.H., Papanastasis, V., Burgess, P.J., Lamersdorf, M., Castro, M., Liagre, F., Rigueiro-Rodríguez, A., 2012. Past, present and future of agroforestry systems in Europe. In: P. Nair, D. Garrity, eds. *Agroforestry-the future of global land use*. Dordrecht: Springer, pp. 285-312. DOI: https://doi.org/10.1007/978-94-007-4676-3_16.

Olivera, A., Fischer, C.R., Bonet, J.A., de Aragón, J., Oliach, D., Colinas, C., 2011. Weed management and irrigation are key treatments in emerging black truffle (*Tuber melanosporum*) cultivation. *New Forests*, 42: 227-239. DOI: https://doi.org/10.1007/s11056-011-9249-9.

Pacioni, G., Leonardi, M., Di Carlo, P., Ranalli, D., Zinni, A., De Laurentiis, G., 2014. Instrumental monitoring of the birth and development of truffles in a *Tuber melanosporum* orchard. *Mycorrhiza*, 24: 65-72. DOI: https://doi.org/10.1007/s00572-014-0561-z.

Ponce, A.R., Águeda, B., Ágreda, T., Modrego, M.P., Aldea, J., Martínez-Peña, F., 2010. Un modelo de potencialidad climática para la trufa negra (*Tuber melanosporum*) en Teruel (España). [A climatic potentiality model for black truffle (*Tuber melanosporum*) in Teruel (Spain)]. *Forest Systems*, 219: 208-220. DOI: https://doi.org/10.5424/fs/2010192-01315.

Repac, I., 2011. Ectomycorrhizal inoculum and inoculation techniques. In: R. Rai, A. Varma, eds. *Diversity and biotechnology of ectomycorrhizae*. Soil Biology series. Berlin: Springer, pp. 43-63. DOI: https://doi.org/10.1007/978-3-642-15196-5_3.

Reyna-Domenech, S., García-Barreda, S., 2009. European black truffle: its potential role in agroforestry development in the marginal lands of Mediterranean calcareous mountains. In: A. Rigueiro-Rodróguez, J. McAdam, M.R. Mosquera-Losada, eds. *Agroforestry in Europe*. Dordrecht: Springer, pp. 295-317. DOI: https://doi.org/10.1007/978-1-4020-8272-6_14.

Rosa-Gruszecka, A., Hilszczańska, D., Gil W., Kosel, B., 2017. Historia i perspektywy użytkowania i badań trufli w Polsce. (History and perspectives of utilisation and research on truffles in Poland). *Sylwan*, 161 (4): 320-327. DOI: https://doi.org/10.26202/sylwan.2017013.

Samils, N., 2002. The socioeconomic impact of truffle cultivation in rural Spain and its potential to encourage pioneer cultivation in Sweden. PHD thesis, SLU Uppsala, Sweden.
Samils, N., Olivera, A., Danell, E., Alexander, S.J., Fischer, C., Colinas, C., 2008. The socioeconomic impact of truffle cultivation in rural Spain. *Economic Botany*, 62 (3): 331. DOI: https://doi.org/10.1007/s12231-008-9030-y.
Shamekh, S., Grebenc, T., Leisola, M., 2014. The cultivation of oak seedlings inoculated with *Tuber aestivum* Vittad. in the boreal region of Finland. *Mycological Progress*, 13: 373-380. DOI: https://doi.org/10.1007/s11557-013-0923-5.
Sourzat, P., Kulifaj, M., Montant, C., 1993. Résultats techniques sur la trufficulture à partir d'expérimentations conduites dans le Lot entre 1985 et 1992. Station d'expérimentation sur la Truffe. GIS Truffe. Le Montat: Lycée Professionnel Agricole de Cahors, 404 pp.
Stöbbe, U., Egli, S., Tegel, W., Peter, M., Sproll, L., Büntgen, U., 2013a. Potential and limitations of Burgundy truffle cultivation. *Applied Microbiology and Biotechnology*, 97: 5215-5224. DOI: https://doi.org/10.1007/s00253-013--4956-0.
Stöbbe, U., Stöbbe, A., Sproll, L., Tegel, W., Peter, M., Büntgen, U., Egli, S., 2013b. New evidence for the symbiosis between *Tuber aestivum* and *Picea abies*. *Mycorrhiza*, 23: 669-673. DOI: https://doi.org/10.1007/s00572-013--0508-9.
Suz, L.M., Martín, M.P., Fischer, C.R., Bonet, J.A., Colinas, C., 2010. Can NPK fertilizers enhance seedling growth and mycorrhizal status of *Tuber melanosporum*-inoculated *Quercus ilex* seedlings? *Mycorrhiza*, 20: 349-360. DOI: https://doi.org/10.1007/s00572-009-0289-3.
Thomas, P.W., 2012. The role of pH in *Tuber aestivum* syn. *uncinatum* mycorrhiza development within commercial orchards. *Acta Mycologica*, 47: 161-167. DOI: https://doi.org/10.5586/am.2012.019.
Thomas, P., Büntgen, U., 2017. First harvest of Périgord black truffle in the UK as a result of climate change. *Climate Research*, 74 (1): 67-70. DOI: https://doi.org/10.3354/cr01494.
Thomas, P., Büntgen, U., 2019. A risk assessment of Europe's black truffle sector under predicted climate change. *Science of The Total Environment*, 655: 27-34. DOI: https://doi.org/10.1016/j.scitotenv.2018.11.252.
Thomas, P.W., Vazquez, L.B., 2022. A novel approach to combine food production with carbon sequestration, biodiversity and conservation goals. *Science of The Total Environment*, 806: 151301. DOI: https://doi.org/10.1016/j.scitotenv.2021.151301.
Urban, A., 2016. Truffles and small mammals. In: A. Zambonelli, M. Iotti, C. Murat, eds. *True truffle (Tuber spp.) in the world*. Cham: Springer, pp. 353-373. DOI: https://doi.org/10.1007/978-3-319-31436-5_21.
Wedén, C., Danell, E., Camacho, F.J., Backlund, A., 2004. The population of the hypogeous fungus *Tuber aestivum* syn. *T. uncinatum* on the island of Gotland. *Mycorrhiza* 14: 19-23. DOI: https://doi.org/10.1007/s00572-003-0271-4.
Wedén, C., Pettersson, L., Danell, E., 2009. Truffle cultivation in Sweden: Results from *Quercus robur* and *Corylus avellana* field trials on the island of Gotland. *Scandinavian Journal of Forest Research*, 24 (1): 37-53. DOI: https://doi.org/10.1080/02827580802562056.
Zambonelli, A., Salomoni, S., Pisi, A., 1993. Caratterizzazione anatomo-morfologica delle micorrize di *Tuber* spp. su *Quercus pubescens* Willd. *Micologia Italiana*, 3: 73-90.
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: 65-73. DOI: https://doi.org/10.1080/01140671.2005.9514332.
Zambonelli, A., Iotti, M., Piattoni, F., 2008. Problems and perspectives in the production of *Tuber* infected plants. Proceedings of the sixth international conference on mushroom biology and mushroom products. 29th September--3rd October 2008, Bonn: GAMU, pp. 263-271. Available from https://www.researchgate.net/profile/Federica--Piattoni-2/publication/357884912_Problems_and_Perspectives_in_the_Production_of_Tuber_Infected_Plants/links/61e583ed8d338833e37689cb/Problems-and-Perspectives-in-the-Production-of-Tuber-Infected-Plants.pdf [accessed: 21.06.2022].
Zambonelli, A., Iotti, M., Barbieri, E., Amicucci, A., Stocchi, V., Peintner, U., Hall, I.R., 2010. The microbial communities and fruiting of edible ectomycorrhizal mushrooms. *Acta Botanica Yunnanica*, 16: 81-85.

STRESZCZENIE

Mykoryzacja truflą burgundzką wybranych gatunków roślin żywicielskich w warunkach szklarniowych

Grzyby z rodzaju *Tuber* (trufla) są najbardziej cenionymi jadalnymi grzybami ektomykoryzowymi. Uprawa trufli została zainicjowana we Francji na początku XIX w. Impulsem do tego był spadek plonowania trufli na stanowiskach naturalnych w drzewostanach. Obecnie najczęściej uprawia-

nymi gatunkami trufli na świecie są trufla czarnozarodnikowa *Tuber melanosporum* Vittad. i trufla burgundzka (syn. trufla letnia) *Tuber aestivum* Vittad.

W Polsce zakładanie plantacji truflowych jest stosunkowo nowym i innowacyjnym przedsięwzięciem naukowo-gospodarczym, wpisującym się w popularyzowany na świecie kierunek agroleśnictwa. Ze względu na dużą plastyczność ekologiczną oraz naturalne występowanie w naszym kraju trufla letnia została wybrana jako gatunek, którego uprawa w Polsce ma duże szanse powodzenia. Innowacyjny charakter tego typu zagospodarowania terenu w Polsce powoduje, że wszelkie badania, w tym dotyczące produkcji mykoryzowanego truflą letnią materiału sadzeniowego, mają charakter pionierski. Celem badań było poznanie roślin żywicielskich, których inokulacja truflą burgundzką charakteryzowałaby się najwyższą skutecznością mykoryzacji. Dodatkowym celem była ocena skuteczności dwóch metod inokulacji w warunkach szklarniowych, różniących się sposobem i czasem aplikacji zarodników *T. aestivum* do podłoża. W toku badań sformułowano dwie hipotezy:

– korzenie drzew liściastych będą skuteczniej (pod względem liczby i czasu) skolonizowane przez *T. aestivum* niż drzewa iglaste;
– zastosowanie inokulum jednocześnie z siewem nasion zapewnia wyższą skuteczność mykoryzacji.

W warunkach szklarniowych przeprowadzono inokulację zarodnikami trufli burgundzkiej 4 gatunków roślin żywicieli: *Pinus sylvestris* L., *Larix decidua* Mill., *Quercus robur* L. i *Fagus sylvatica* L. Inokulację przeprowadzono w dwóch terminach: w trakcie wysiewu nasion i 4 tygodnie po nim. Sadzonki przebywały w warunkach kontrolowanych przez 11 miesięcy. Po 3, 6 i 11 miesiącach wzrostu pobrano z wybranych sadzonek drobne korzenie w celu sprawdzenia skuteczności mykoryzacji. Określono ją na podstawie cech morfologicznych korzeni i oceniono w 4-stopniowej skali na podstawie udziału korzeni z mykoryzami *T. aestivum*: 0-25% – stopień 1, 26-50% – stopień 2, 51-75% – stopień 3, 76-100% – stopień 4.

Na podstawie uzyskanych wyników stwierdzono, że czas aplikacji zarodników *T. aestivum* nie miał istotnego wpływu na skuteczność mykoryzacji, niezależnie od gatunku rośliny żywiciela (ryc. 1). Szybkość mykoryzacji siewek modrzewia, sosny, buka i dębu przez *T. aestivum* nie różniła się istotnie (czas i liczba) (ryc. 2-6, tab. 1). Sadzonki dębu charakteryzowały się jednak wyższym wskaźnikiem mykoryzacji, co tłumaczy popularność wykorzystania tego gatunku do zakładania plantacji trufli burgundzkiej.