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Grażyna Kłobus, Faculty of Biological Sciences, University of Wrocław, Poland

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Competing interests

No competing interests have been declared.

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ORIGINAL RESEARCH PAPER

Arum-type of arbuscular mycorrhizae, dark septate endophytes and *Olpidium* spp. in fine roots of container-grown seedlings of *Sorbus torminalis* (Rosaceae)

Roman M. Bzdyk¹, Josef Kohler^{2,3}, Jacek Olchowik⁴, Marta Aleksandrowicz-Trzcńska⁴, Thomas Kirisits^{5*}¹ Department of Forest Protection, Forest Research Institute, Braci Leśnej 3, 05-090 Sękocin Stary, Poland² Plant Ecology, Institute of Biology, Freie Universität Berlin, 141095 Berlin, Germany³ Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), 14195 Berlin, Germany⁴ Department of Forest Protection and Ecology, Faculty of Forestry, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland⁵ Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences, Vienna (BOKU), 1190 Vienna, Austria* Corresponding author. Email: thomas.kirisits@boku.ac.at**Abstract**

The aim of this study was to determine the mycorrhizal status of nursery seedlings of the wild service tree (*Sorbus torminalis*), which belongs to the Rosaceae family. Its mycorrhizal associations are still fragmentarily known, and data from the few existing studies indicate that it forms ectomycorrhizal symbiosis (ECM). We analyzed the degree of mycorrhizal colonization of thirty 2-year-old container-grown *S. torminalis* nursery seedlings, which belonged to three single-tree progenies. The roots were dominated by arbuscular mycorrhizae (AM), with the morphology of the *Arum*-type containing arbuscules, vesicles and hyphae; however, no ECM structures were found. The degree of root colonization of the analyzed seedlings by AM fungi was 83.6% and did not differ significantly between the three single-tree progenies. In addition to AM, structures of dark septate endophytes (0.7%) and sporangia of *Olpidium* spp. (1.1%) were found in wild service tree roots. In agreement with previous studies, we confirmed arbuscular mycorrhizae for *S. torminalis*. Moreover, this is the first report that roots of this *Sorbus* species show the *Arum*-type morphology of AM and are associated with *Olpidium* species.

Keywords

wild service tree; arbuscular mycorrhizal fungi (AMF); mycorrhizal status; morphological types; root endobionts; symbiosis

Introduction

Wild service tree [*Sorbus torminalis* (L.) Crantz], also known as chequer tree or checker tree, belongs to the Rosaceae family and occurs in Western, Southern, and Central Europe as well as in parts of Western Asia (Asia Minor, Caucasus) [1]. It is mainly a minor component of various oak-dominated forests and occurs throughout its range at low densities, either as individual tree or in small groups of up to 30 trees per hectare [2,3]. *Sorbus torminalis* grows best on fresh, deep and fertile soils, but its ecological amplitude is wide, and it can thrive on both acid and base-rich soils, at a pH

range between 3.5 and 8 [1]. It is a light demanding species but can persist for some time in half-shady conditions under the canopy of other tree species. However, on fresh and fertile sites it can hardly keep up with the growth of the dominant tree species, especially with European beech (*Fagus sylvatica* L.), and is therefore often out-competed. In contrast, it is more competitive on warm-dry, often calcareous sites with shallow soils up to 900 meters above sea level, especially in light oak, oak-hornbeam and pine forests [1,4].

Wild service tree is a valuable noble hardwood species. Highly recommended for the furniture and veneer industry, for turnery and for the construction of musical and measuring instruments, the interest in the use of *S. torminalis* in forestry has been increasing in the last four decades in countries such as Austria, Germany, France, Switzerland, Romania, and Italy [1,3,4]. The fruits are used to produce liquors, especially in Germany and Austria [3–5].

Although wild service tree is a relatively rare species throughout its distribution range, it is managed as forest and fruit tree in parts of Austria, Germany, France, and other European countries, resulting in the production of valuable niche products such as veneer logs and exquisite schnapps [4,5]. At the species' northern distribution limit in Denmark, where its reproductive success under current climatic conditions is reduced [6], wild service tree is considered as an interesting tree species for the future, to increase biodiversity as well as for aesthetic and economic reasons [7]. In Poland, *S. torminalis* is one of the rarest woody plants, increases the biodiversity of pine-oak and oak forests and is a valuable species protected by law [8,9]. Since 2005 programs of reintroduction of wild service tree have been initiated in some Polish forest districts [10,11]. Bednorz [9] proposed a conservation program for Polish forests, where gene reserves and protected trees would be sources of seeds used for propagation in selected nurseries, and in turn, these seedlings would be planted on suitable sites, where the species is currently absent.

Knowledge on the association of *S. torminalis* with other organisms can be of practical importance, in order to optimize the sustainable management and conservation of the species. Kirisits and Wegensteiner [12] suggested targeted studies on diseases, pests and abiotic factors affecting wild service tree, as an increase in the cultivation and use of the species bears the risk that damaging agents become more important. A more thorough understanding on symbiotic interactions of *S. torminalis* with other species is also desirable. For example, it is important to know the mycorrhizal status of nursery seedlings, because compatible mycorrhizal associates can improve their establishment and growth.

Tree species in the Rosaceae family are usually considered to be associated mainly with ectomycorrhizal (ECM) fungi and to a lesser degree with arbuscular mycorrhizal (AM) fungi [13]. Within the family there are, however, great differences between genera and species regarding the occurrence and relative importance of these two major types of mycorrhizae. Species in the genus *Sorbus* are normally associated with AM fungi and rarely with ECM fungi [14,15]. Whitebeam (*Sorbus aria* Crantz) and mountain ash (*Sorbus aucuparia* L.) form AM or ECM, or remain nonmycorrhizal (NM) [14]. A few studies reported that *S. torminalis* forms ectomycorrhizae [14,16,17]; however, it was not documented which ECM species were involved in this symbiosis. In contrast, according to recent investigations [18,19] and a recent review [20] wild service tree has primarily AM, but also ECM. Thus, reports of the mycorrhizal status of *S. torminalis* are contradictory.

It is well known that AM and ECM fungi as well as some strains of dark septate endophytes (DSE) – another group of symbionts of plants [21] – can stimulate the growth and vitality of plants [22]; therefore, an understanding of the occurrence and frequency of these fungal associates may facilitate the maintenance, conservation and sustainable management of the rare resources of wild service tree. Besides, roots of plants are colonized by other endophytes including *Olpidium* species. *Olpidium* spp. are common biotrophic, symptomless root parasites of many vascular plants; they are also known as vectors of plant viruses [23]. Thus, the aim of this study was to determine the mycorrhizal status of *S. torminalis* and its degree of mycorrhizal colonization by examining 2-year-old container-grown seedlings from a forest nursery. Furthermore, we investigated whether other fungal root endobionts such as DSE or *Olpidium* species colonized the fine roots of the wild service tree seedlings.

Material and methods

Sorbus torminalis seedlings

Thirty 2-year-old container-grown *S. torminalis* seedlings, raised at the forest experimental garden and nursery “Knödelhütte” [Institute of Silviculture, University of Natural Resources and Life Sciences, Vienna (BOKU), Austria] in Vienna, were examined in this study. The seedlings originated from three different parent trees (labeled as KH6, KH10, and KH11) of the *S. torminalis* collection in the forest experimental garden and thus represented single-tree progenies, with 10 seedlings per parent tree. A ready mix potting soil for woody and perennial plants (Terra Vita Pflanzsubstrat, T6-Gehölze/Stauden; Franz KRANZINGER GmbH, Straßwalchen, Austria) with a pH ranging from 6.3 to 6.8 was used as substrate to grow the plants. At the end of June 2013 the seedlings were placed in the garden of the Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF) and regularly watered according to weather conditions. At this time, the height, root collar diameter, and stem diameter (at 10 cm distance from the soil surface) of each seedling were measured (Tab. 1).

Root sampling and assessment of fungal colonization

In order to analyze the type of mycorrhizae, seedling roots and surrounding soil were carefully taken from the pots, and transported in properly labeled plastic bags to the laboratory. Sampling was done successively at 10 dates during a period from 10 July to 23 August 2013. At each date, samples from one seedling of each single-tree progeny were collected. For determination of fungal colonization, we used the staining method described by Vierheilig et al. [24]. Randomly, nonlignified 1- to 2-cm-long root pieces (each from a different root) were cut out using a scalpel, placed in a special sieve and purified from soil particles under tap water. To remove secondary metabolites (tannins, phenols, suberin, and lignin) of the root cell walls, the roots were cleared in 10% KOH solution at 90°C for 20 min. Before staining, the roots were rinsed 3 times in water with a few drops of 5% acetic acid. Then, they were stained with 5% acetic acid in a mixture of 5% Pelikan Ink Blue®, by boiling them for 5 min. In order to remove excess ink from the root tissues, root samples were washed 3 times and then stored for 30 min in water containing a few drops of acetic acid [24]. The entire staining procedure was carried out at room temperature.

The prepared roots were first analyzed under a stereomicroscope (Wild M3C, Switzerland), and then viewed with a dissecting light microscope (Carl Zeiss Axiophot Photomikroskop, Germany). In a first step, roots were examined in a small amount of water in a Petri dish under the stereomicroscope at 10–40× magnification. As these observations did not reveal the characteristic structures of ECM (mantle, extraradical hyphae), the same roots were examined under the dissecting light microscope (at 400× and 1000× magnifications). The degree of arbuscular mycorrhization and

Tab. 1 Biometrical parameters (means) of two-year-old container-grown *S. torminalis* seedlings (three single-tree progenies labeled as KH6, KH10, and KH11) from the forest experimental garden and nursery “Knödelhütte” in Vienna (Austria) investigated in this study.

Single-tree progeny	No. of examined seedlings	Seedling height (cm) ^a	Root collar diameter (mm) ^b	Stem diameter at 10 cm distance from the soil (mm) ^b
KH6	10	73.4a	8.7a	6.6a
KH10	10	65.9a	6.5b	5.8b
KH11	10	67.1a	6.9b	5.9b

^a Mean values with the same letter within the column were not significantly different ($p > 0.05$) according to the Kruskal–Wallis test. ^b Mean values with different letters within the column were significantly different ($p < 0.05$) according to the Kruskal–Wallis test, followed by pairwise Mann–Whitney U tests.

colonization of other fungi were determined according to the protocol described by McGonigle et al. [25]. For this purpose, ten randomly selected root pieces from each seedling (100 per single-tree progeny and 300 in total for all 30 seedlings) were mounted on slides after staining (see above), and on 10 randomly gridline intersects per root sample (100 per seedling, 1000 per single-tree progeny, and 3000 in total for all 30 seedlings) the following characteristics were recorded: presence and absence of AM structures (arbuscules, vesicles, *Arum*-type AM morphology), the occurrence of DSE and fungi from the genus *Olpidium*, points of infection, or absence of fungal colonization [22,26]. The arbuscular mycorrhizal colonization types observed in roots were specified according to Dickson's [26] classification. DSE were identified based on the presence of regularly septate hyphae, which were usually darkly pigmented, with sclerotia occurring facultatively [21]. Sporangia of the genus *Olpidium* (F_{Olp}) were determined according to Agrios [27]. Each inspected location was photographed, and the data described above was recorded.

The degree of mycorrhization (M) of a root was defined as the number of observations which were characteristic for arbuscular mycorrhizae in relation to all examined locations and multiplied by 100. In the same way, the frequency of occurrence of arbuscules (A), vesicles with coarse hyphae (V) and hyphae (H), as well dark septate endophytes (DSE) and *Olpidium* spp. (Olp) in a root was determined. In addition, the percentage of plants colonized by arbuscular mycorrhizal fungi (AMF; F_{AMF}), DSE (F_{DSE}), and *Olpidium* spp. (F_{Olp}) was calculated (when structures characteristic for AMF, DSE, or *Olpidium* spp. were found in at least one root per seedling, the seedling was scored positive for colonization by these fungi).

Statistical analyses

Data were checked for normal distribution and homogeneity of variance using the Shapiro–Wilk test and the Levene test, respectively. Differences in the biometric data among the three single-tree progenies of *S. torminalis* were tested with the Kruskal–Wallis test. If this test indicated significant differences, the single-tree progenies were pairwise compared with the Mann–Whitney *U* test. For percentages of parameters referring to fungal colonization of roots mean values of observations on each seedling were used in the statistical analyses. This data was arcsine transformed for the analyses, in order to fulfill the requirements of parametric statistical testing. Differences in the degree of AM fungal colonization of roots and in the frequency of various fungal structures between the single-tree *S. torminalis* progenies were tested with one-way analyses of variance (ANOVA), followed by the Tukey test, in the case the ANOVA revealed significant differences. Spearman's rank correlations were performed between the biometric parameters of the seedlings and the degree of colonization of AMF, DSE, and *Olpidium* spp. The data were analyzed using STATISTICA software, version 12 (StatSoft Inc., Tulsa, USA, 2015; <http://www.statsoft.com>).

Results

Arbuscular mycorrhizal fungi

The analysis of roots of 30 container-grown seedlings of *S. torminalis* from a nursery in Vienna (Austria) revealed only the presence of AM (endomycorrhizae). In contrast, we did not find a mantle and a Hartig net (the characteristic structures of ectomycorrhizae) in any of the examined *S. torminalis* roots (Fig. 1a). Typical AM structures (Fig. 1b–d) were observed in the fine roots of all seedlings. The mean AM fungal colonization rate for all examined roots amounted 83.6%, with a high abundance of hyphae ($H = 38.3\%$) and arbuscules ($A = 38.2\%$), but a low abundance of vesicles ($V = 5\%$). The lowest rate of mycorrhization was found in progeny KH10 ($M = 80.8\%$), followed by KH6 (82.1%) and KH11 (83.7%); differences were, however, not statistically significant (Tab. 2).

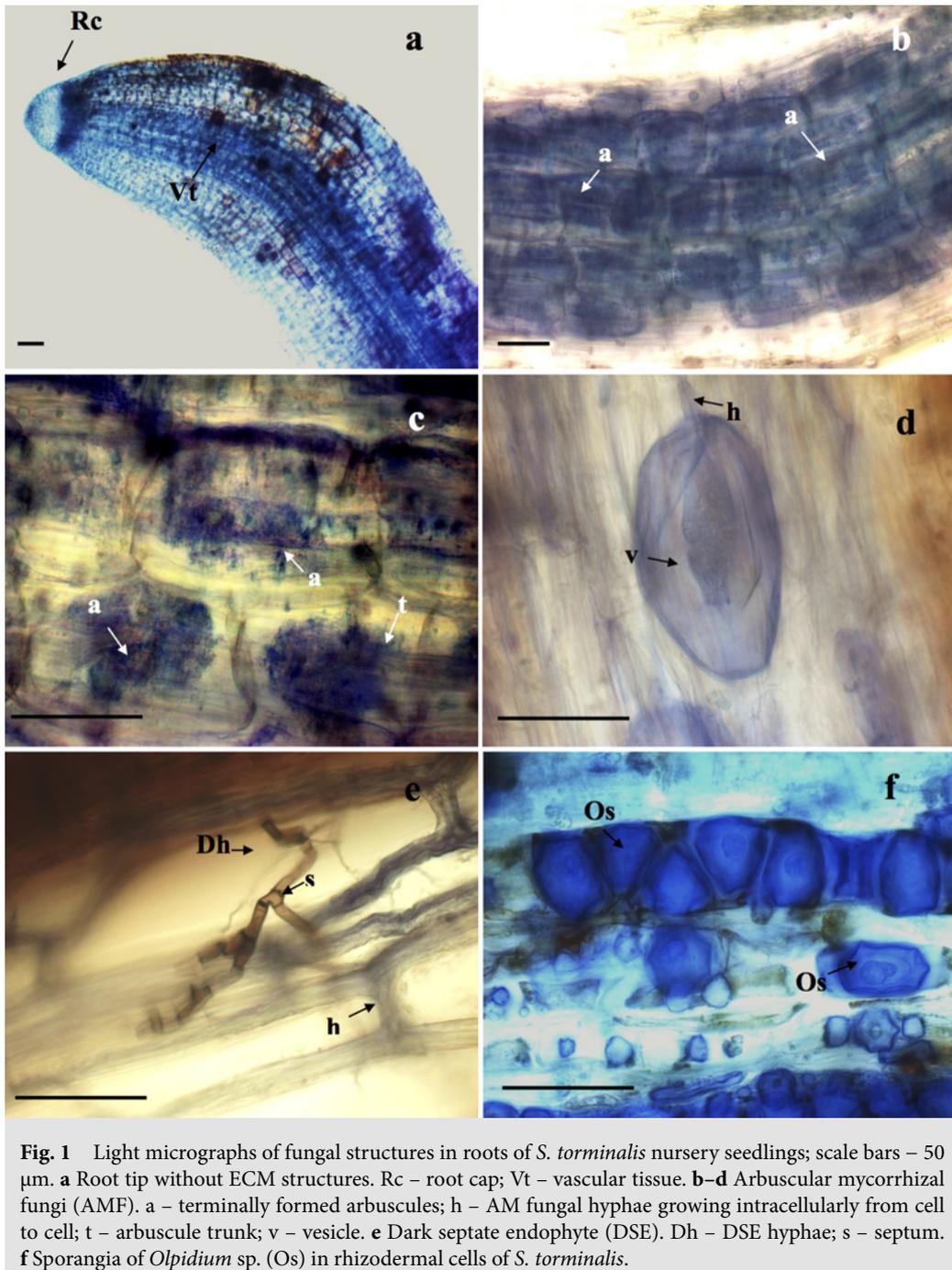


Fig. 1 Light micrographs of fungal structures in roots of *S. torminalis* nursery seedlings; scale bars – 50 μ m. **a** Root tip without ECM structures. Rc – root cap; Vt – vascular tissue. **b–d** Arbuscular mycorrhizal fungi (AMF). a – terminally formed arbuscules; h – AM fungal hyphae growing intracellularly from cell to cell; t – arbuscule trunk; v – vesicle. **e** Dark septate endophyte (DSE). Dh – DSE hyphae; s – septum. **f** Sporangia of *Olpidium* sp. (Os) in rhizodermal cells of *S. torminalis*.

Arbuscules and hyphae occurred in all examined trees, whereas vesicles were observed less frequently, in 76.7% of the seedlings. All examined plants showed the *Arum*-type morphology of AM, and according to Dickson's [26] classification four morphotypes were detected: *Arum* A, *Arum* 1 (A1), and intermediate types 1 (I1) and 3 (I3). In addition, in the surrounding soil spores and a mycelial network around the roots were found (detailed data not shown).

Dark septate endophytes and *Olpidium* spp.

In addition to the characteristic AM structures, DSE hyphae and sporangia of *Olpidium* species were detected in the *S. torminalis* roots. Single brownish or, rarely, stained with blue ink DSE hyphae of varying diameters were found in the outer cortex and rhizodermis (Fig. 1e). Single DSE hyphae were also detected on the root surface. *Olpidium* sporangia, which stained with blue ink, were found inside root epidermal

Tab. 2 Arbuscular mycorrhization (AM) and fungal root endophyte associations of two-year-old container-grown *S. torminalis* seedlings (three single-tree progenies labeled as KH6, KH10, and KH11) from the forest experimental garden and nursery “Knödelhütte” in Vienna (Austria).

Single-tree progeny	AM type ^a	Arbuscular mycorrhizal fungi ^b (%), mean ± standard deviation of values from 10 seedlings per single tree progeny				Other root endophytes ^c (%), <i>n</i> = 10 seedlings per single-tree progeny)	
		M	A	V	H	F _{DSE}	F _{Olp}
KH6	<i>Arum</i>	82.1 ± 14.6a	31.8 ± 18.4a	3.0 ± 3.9a	47.1 ± 19.9a	40	10
KH10	<i>Arum</i>	80.8 ± 12.9a	38.0 ± 14.1a	7.6 ± 8.9a	35.2 ± 20.4a	30	60
KH11	<i>Arum</i>	83.7 ± 12.1a	44.7 ± 6.8a	4.5 ± 4.1a	34.4 ± 14.2a	0	40

Mean percentages with different letters within columns were significantly different ($p < 0.05$) according to one-way analyses of variance (ANOVA) followed by the Tukey test. ^a Arbuscular mycorrhizal (AM) status and AM morphotype (according to Dickson [26]). ^b Degree of mycorrhization (M; percent/portion of a root colonized ±SD), frequency of occurrence of arbuscules (A), vesicles (V) with hyphae and hyphae (H; percent/portion of a root with these structures ±SD) ^c Frequency of occurrence (percent of plants colonized) of other fungal root endophytes. F_{DSE} – hyphae of dark septate endophytes (DSE); F_{Olp} – sporangia of *Olpidium* spp.

cells (Fig. 1f). DSE (total F_{DSE} = 23.3%) and *Olpidium* sporangia (total F_{Olp} = 36.7%) occurred at lower frequencies in seedlings than AM fungi (which were found in all 30 investigated plants). While *Olpidium* sporangia were recorded in all three single-tree progenies, DSE were not found in any seedling of progeny KH11 (Tab. 2). The overall root colonization rate of these fungi was low (DSE = 0.7%, Olp = 1.1%).

Characteristics of the seedlings and relationships with root endophytes

During the entire observation period the investigated wild service tree plants looked vigorous, and no obvious damage was recorded on them. Seedlings of the three single-tree progenies did not differ significantly in height; however, significant differences in the diameter of the root collar and stem diameter at 10 cm distance from the soil were detected (Tab. 1). Spearman's rank correlation showed a significant, moderately positive correlation between the AMF colonization rate and the root collar diameter of the seedlings ($r = 0.46$, $p = 0.011$). A significant, moderately negative correlation was detected between the colonization rate of *Olpidium* spp. and the root collar diameter of the plants ($r = -0.39$, $p = 0.034$).

Discussion

Many reports indicate the consistent occurrence of the same type of mycorrhizae, either ECM or AM, or the lack of mycorrhizae, on plants which belong to the same genus or family [16,22,28]. This applies in particular to plant families associated with ECM (e.g., Fagaceae or Pinaceae) and those containing species which are usually non-mycorrhizal, e.g., *Castanopsis indica* (Roxb.) A.DC. There are, however, many exceptions to this pattern [14].

There are several tree species, for example *Populus* spp., that often are associated with both types of mycorrhizae, AM and ECM [29]. Tree species belonging to the Pinaceae [e.g., *Pseudotsuga menziesii* (Mirb.) Franco, *Abies lasiocarpa* (Hook.) Nutt.], which usually have ECM, can also be associated with endomycorrhizae, possessing fungal hyphae and vesicles, but lacking arbuscules [30]. Many species of shrubs and trees; e.g., willow (*Salix* spp.), linden (*Tilia* spp.), and mountain ash (*S. aucuparia*) are known to host both major types of mycorrhizae, AM and ECM [14,31]. In the case of *S. aucuparia*, it depends on the habitat: in a boreal environment with light soil both mycorrhizal types were recorded, whereas in swamp alder woods AM dominated [31].

The supposed association of *S. torminalis* with ECM [14,16,17,20] is in clear contrast to our investigations and those of others [18,19], in which wild service tree was found to be associated with AM fungi. Harley and Harley [16] listed the majority of known mycorrhizal associations of plants of the British flora, and in recent reviews new information was added [14,20]. For the status of *S. torminalis* as being ectomycorrhizal, all three reviews refer to an original report by the Polish phytopathologist Truszkowska [31]. Truszkowska [31] studied mycotrophism of swamp alder woods and indicated details for the genus *Sorbus* only for mountain ash (*S. aucuparia*), which shows both AM and ECM associations. However, she did not mention *S. torminalis* at all. This fact was insufficiently explained by Harley and Harley [16], and subsequent publications took over the unsubstantiated association of *S. torminalis* with ECM [14,20].

Another description of the mycorrhizal status of *S. torminalis* was provided by Akhmetzhanova et al. [17], who translated, modernized and converted the doctoral thesis of Selivanov (1976; not seen), previously only available as a printed version in Russian, into digital form. This study covered the period from 1957 to 1975 and resulted in an extensive database (7445 individual plant specimens of 2970 plant species) with types and degree of mycorrhization of plants from the former Soviet Union (USSR). However, in the entire database, only one specimen of wild service tree was included, based on which this tree species was assigned to be associated with ECM (Akhmetzhanova et al. [17], following Selivanov, 1976). Based on one specimen, it is virtually impossible to clearly characterize the type of mycorrhization. Moreover, in the case of mature trees the second and third lateral root samples were collected from a depth of 5–20 cm, so that it is uncertain whether the sampled roots were indeed from *S. torminalis*. Therefore, we can conclude that the quoted literature up to now does not provide sufficient information on the ECM associations of wild service tree.

In the roots of *S. torminalis* seedlings analyzed in the present study, AM structures (arbuscules and vesicles) were found consistently and at varying frequencies. Thus, we unambiguously observed arbuscular mycorrhizae in the *S. torminalis* seedlings, but after careful inspection of roots under the stereomicroscope and dissecting light microscope we could not find the characteristic structures of ECM. In the present study, the total rate of mycorrhization was 83.6%, which appears to be higher than that on the closely related *S. aucuparia*, in which the degree of AMF colonization under natural conditions amounted 10–20% [32], 13–40% [33], or 30–60% [34]. However, as reviewed by Harley and Harley [16], this tree species forms both AM and ECM, which may explain the lower AM colonization degree compared to that we detected in *S. torminalis*. Previously, Azcón-Aguilar et al. [18] showed that *S. torminalis* forms AM in natural environments in the Sierra Nevada in Spain; however, in this study mycorrhizal colonization of roots, ranging from 1 to 10%, was low. Likewise, Moradi et al. [19] inferred that *S. torminalis* has a strong symbiosis with AMF, with a mycorrhizal colonization of up to 68%, in natural mixed forests in northern Iran. In this study, AMF colonization rates of wild service tree roots were affected by season (on an average about 51% in spring and 32% in autumn). As in our study, ECM colonization of *S. torminalis* roots was not detected by Azcón-Aguilar et al. [18] and Moradi et al. [19]. We found a significant positive correlation between the degree of AM colonization and the root collar diameter of the seedlings ($r = 0.46$). A positive effect of AMF on plant growth has been previously documented in numerous studies (e.g., [35–37]).

According to Dickson's [26] classification, four AM morphotypes were found in the roots of the wild service tree seedlings: *Arum* A, *Arum* 1 (A1), and intermediate types 1 (I1) and 3 (I3). It is thought that the presence of a particular morphotype is strongly related to the morphological and physiological characteristics of roots, which may favor the formation of one or the other type. According to Read and Smith [22], more plant families have been recorded forming *Paris*-types with intracellular coils than *Arum*-types with characteristic arbuscules. However, our work showed only the *Arum*-type morphology of AM in the analyzed seedlings. Although Azcón-Aguilar et al. [18] and Moradi et al. [19] documented AM for *S. torminalis*, they did not mention the morphotypes of AMF colonization in roots of this tree species. To our knowledge, this is therefore the first report of occurrence of *Arum*-type arbuscular mycorrhizae in *S. torminalis* roots.

As we examined two-year-old potted seedlings, it is necessary to bear the extramatrical mycelium associated with mature trees and the age of the host plant as important factors in the formation of ECM associations in mind. The extramatrical mycelium connected with roots of mature trees can be a source of inoculum, leading to colonization of young plants with ECM fungi. For naturally regenerated *S. torminalis* seedlings in the forest growing in close proximity to mature trees, a richer source of inoculum and thereby a richer composition of ECM fungi, resulting in a higher probability of formation of ECM, can be expected [38,39]. Some species of ECM are unable to colonize young plants, but are only specialized in the colonization of mature trees [40,41]. This may suggest that the age of a plant can have a crucial impact on the root colonization by ECM species. Thus, one could expect the occurrence of ECM fungi on older wild service trees, particularly on forest sites. However, neither naturally occurring *S. torminalis* trees in the Spanish Sierra Nevada [18] nor trees in mixed forests in northern Iran [19] were found to form ECM, which may suggest that *S. torminalis* is indeed only associated with AMF.

In Austria, fruiting bodies of various basidiomycetes forming ectomycorrhizae [*Boletus aestivalis* (Paulet) Fr., *Boletus edulis* Bull., *Boletus fechtneri* Velen, *Boletus pinophilus* Pilát & Dermek, and *Tricholoma fracticum* (Britz.) Kreisel] were observed adjacent to wild service trees on forest sites [42,43]. This could indicate (but certainly does not prove) that this tree species is also associated with ECM fungi. The chance of mistakes resulting from circumstantially connecting fruiting bodies with presumed host plants growing nearby is particularly high when fruiting bodies are large distances apart from the true host plant or when a plant is pre-assumed to have ECM associations, as it was the case with *S. torminalis* [16,44]. Many older studies of this type have, with hindsight, been proven to be wrong, for example for trees in the genera *Acer*, *Fraxinus*, or *Ulmus*, which are now known not to be associated with ECM fungi [13]. Thus, further investigations are necessary in order to definitely clarify whether or not *S. torminalis* forms ECM.

Sorbus torminalis roots were also colonized by dark septate endophytes (DSE), but only at a low degree (0.7%) and in co-occurrence with AM fungi. When calculated for entire seedlings, single-tree progenies KH6 and KH10 showed a frequency of association with DSE of 30% and 40%, respectively, whereas in none of the roots of progeny KH11 DSE hyphae were detected (Tab. 1). The overall colonization frequency of *S. torminalis* seedlings by DSE ($F_{DSE} = 23.3\%$) was similar compared to that in a study on fungal root endophyte associations of various forest tree species, where the frequency of DSE ranged from 11–28% (e.g., in *Fagus sylvatica*) to 58–87% (e.g., in *Pinus sylvestris* L.) and 57–90% [e.g., in *Picea abies* (L.) H. Karst.] [45]. Azcón-Aguilar et al. [18] reported DSE colonization rates between 1 and 10% in *S. torminalis* roots in the tree's natural habitat, which are higher than in our study.

Dark septate endophytes are a miscellaneous group of fungi, which colonize plant roots, form symbiosis with conifers and deciduous trees [45,46], and have many features in common with ECM, particularly the formation of a thin fungal mantle and a Hartig net (not found in the present study), which have also been described as features of ectendomycorrhizae [21]. However, in the case of ectendomycorrhizae, hyphae penetrate cortical root tissues not only intercellularly, but also intracellularly [45]. Based on the morphology of such associations, some studies described certain DSE fungi, e.g., *Phialophora finlandica* C.J.K. Wang & H.E. Wilcox on pine, as ectomycorrhizal [47]. The function of DSE in roots of plants is not clear; these associations can be pathogenic or saprotrophic, mutualistic or neutral [21]. DSE are considered as facultative biotrophs rather than as mycorrhizal fungi [22]. A recent meta-analysis of plant responses to dark septate root endophytes indicated that they do not have negative effects on plant performance [48]. Some positive effects on total biomass were shown, and an increase of nitrogen and phosphorus contents in shoots of infected plants occurred [48]. Moreover, DSE appear to enhance plant performance under controlled conditions, when nitrogen is available in organic form.

In the present study, structures characteristic for *Olpidium* spp. were recorded at low frequencies ($Olp = 1.1\%$) in *S. torminalis* roots. Based on the percentage of colonized seedlings, their incidence was, however, quite high and ranged from 10 to 60% in the three single-tree progenies. This is higher than in a study on fungal root endophyte associations of endemic plant species in the Pamir Alay Mountains in Asia

[49] where the frequency of *Olpidium* spp. ranged from 0 to 10%. Species in the genus *Olpidium* belong to the class Chytridiomycetes; thus, they produce zoospores and are obligatorily biotrophic organisms associated with plants as asymptomatic root parasites, and are therefore considered as microorganisms of minor or no importance on plant growth [23,50]. We found a significant negative correlation between *Olpidium* colonization rates and root collar diameter of the seedlings ($r = -0.39$), possibly indicating a negative effect of these fungi on the plants. *Olpidium* species are known as vectors of many pathogenic viruses on cereals, salad, and tobacco [27]. Palle et al. [51] showed that an *Olpidium*-like fungus is associated with roots of symptomatic citrus trees, and detected *Citrus psorosis* virus (cause of *Citrus psorosis* disease) in zoospores from infected roots. In addition, *Olpidium* species have been found to infect many different cultivated plants, including cucumber, lettuce, carrot, and brassicas such as broccoli and cabbage [52], at the same time causing damping off [53]. Although the wild service tree seedlings in this study did not show any disease symptoms, it should be considered that *Olpidium* spp. may be important in terms of their function as vectors of pathogenic viruses [54,55].

In conclusion, this study confirmed arbuscular mycorrhizae for *S. torminalis*, and it reports, for the first time, the *Arum*-type morphology of AM colonization in roots of this *Sorbus* species. Moreover, it represents the first report of *Olpidium* species and confirmed the presence of dark septate endopyhtes in *S. torminalis* roots. Based on our results and that of previous studies carried out in Iran and Spain [18,19] and based on the sparse and partly incorrect reports of ECM associations with wild service tree [14,16,17,20], we propose to regard *S. torminalis* primarily as arbuscular mycorrhizal plant species. However, it should be clarified in future investigations whether this tree species can additionally also form ECM, particularly in natural environments in its temperate distribution range in Central Europe. The information provided in the present could be useful to improve in situ and ex situ conservation efforts for and sustainable management of the rare and valuable *S. torminalis*, because a high degree of mycorrhization can facilitate plant establishment and artificial inoculation of AM fungi may improve plant performance, especially on extreme and disturbed restoration sites.

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