Ectomycorrhizal status of *Larix decidua*-, *Picea abies*- and *Pinus cembra*-nursery plants in South Tyrol

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Abstract

Most European forest trees live in symbiosis with ectomycorrhizal (EM) fungi. Nevertheless, relatively little is known about the mycobiont (fungal EM-partner) species composition and abundances of nursery plants. In this study we therefore investigate EM symbiosis partner of *Larix decidua*, *Picea abies* and *Pinus cembra* seedlings and plantlets from six forest nurseries in South Tyrol (Aicha/Aica, Prad/Prato, Prettau/Predoi, Radein/Redagno, Ulten/Ultimo, Welsberg/Monguelfo). We were interested in dominant mycobionts of each host plant, and if there were differences in species richness and composition between seedling provenances of one host plant species, between different plant species, between nurseries and between season or plant age.

Sampling was performed in autumn 2006 and in spring 2007. Mycorrhized root tips were sorted into EM morphotypes (MTs), and rDNA ITS sequences were generated for several representatives of each MT. Thus, analysed 28,290 root tips and detected a total of 39 MT.

The rate of mycorrhization was 100% in autumn and about 97% in spring in all nurseries and on all plant species. The lower mycorrhization rates in spring were probably caused by the strong growth impulse of plants during this period. The three host plants differed significantly from each other in species composition. The latter was found to be nursery-specific for *Larix decidua* and *Pinus cembra*. Seedling provenance was never significant for mycobiont species composition in nursery plants. However, species composition changed with plant age or season: the *Wilcoxina mikolae* complex dominated on seedlings, being later replaced either by host specific mycobionts, or by mycobionts with a long-distance distribution type.

We detected 14 fungal mycobiont species associated with *Larix decidua*. *Wilcoxina mikolae* and several host-specific *Suillus spp.* (*S. aeruginascens, S. grevillei, S. tridentinus*) were found in all three nurseries. The species composition was nursery specific. *Picea abies* root tips were associated with three fungal species only: *Wilcoxina* sp., *Tuber puberulum*, and *Amphinema byssoides*. The two analysed nurseries did not differ from each other in mycobiont species composition nor in relative abundances. Similar to *Larix,* also *Pinus cembra* nursery plants exhibited a high mycobiont species richness (12 *spp.*). Nurseries did not differ significantly from each other in the number of mycobiont species, but species composition was partly different. *Wilcoxina* sp. and *Pinus cembra* - specific *Suillus spp.* (*S. placidus, S. plorans, S. sibiricus*) dominated in all nurseries.

Although partly being raised in the same nursery, the three host plants *L. decidua*, *Picea abies* and *Pinus cembra* differed significantly in mycobiont species richness and species composition. The mycobiont species composition was specific for each plant: *Amphinema byssoides* and *Wilcoxina* sp. 1 were the only taxa detected on two hosts plant species, *Picea abies* and *Pinus cembra*. The mycorrhizal communities of *L. decidua* and *Pinus cembra* were characterised by the occurrence of host specific fungi (*Suillus spp.*).

Key words: ectomycorrhiza, nursery, *Suillus*, *Wilcoxina*, morphotype, PCR

Zusammenfassung

Obwohl viele europäische Waldbäume in Symbiose mit Ektomykorrhizapilzen (EM) leben, ist relativ wenig über die Zusammensetzung und Abundanzen der Pilzpartner von Forstgartenpflanzen bekannt. Im Laufe dieser Arbeit wurden daher die EM Symbiosepartner von *Larix decidua*, *Picea abies* und *Pinus cembra* (Sämlinge und Pflänzchen) aus sechs Forstgärten Südtirols untersucht (Aicha/Aica, Prad/Prato, Prettau/Predoi, Radein/Redagno, Ulten/Ultimo, Welsberg/Monguelfo). Ein Ziel dieser Studie war es, die dominierenden Symbiosepartner dieser drei Wirtspflanzen zu identifizieren und zu vergleichen. Weiters waren wir daran interessiert, ob es Unterschiede im Artenreichtum und der Artenzusammensetzung zwischen Pflanzen verschiedener Forstgärten, verschiedener Provenienzen, oder verschiedenen Alters gibt.
Die Probennahme wurde im Herbst 2006 und im Frühling 2007 durchgeführt. Die mykorrhizierten Wurzelspitzen wurden in EM Morphotypen (MT) unterteilt und der rDNA ITS Abschnitt von mehreren Wurzelspitzen eines MT´s wurde sequenziert. Für diese Untersuchung wurden insgesamt 28.290 Wurzelspitzen analysiert, und 39 MT identifiziert. Die Mykorrhizierungsrate war generell hoch und lag im Herbst bei 100%, im Frühling bei 97%. Die niedrigere Mykorrhizierungsrate im Frühling lässt sich durch den starken Wachstumsimpuls der Pflanzen während dieser Jahreszeit erklären. Die Artenzusammensetzung war spezifisch für Pflanzenarten, und bei *L. decidua* und *Pinus cembra* auch Forstgarten spezifisch. Die Samenprovenienz war nie signifikant für die Zusammensetzung der Symbiosepartner im Pflanzgarten. Aber die Artenzusammensetzung änderte sich mit dem Alter der Pflanzen bzw. mit der Jahreszeit: Symbiosepartner aus dem *Wilcoxina mikoale*-Komplex dominierten auf Sämlingen, und wurden aber bald entweder durch wirtsspezifische Pilze, oder durch Pilze mit einem Langstrecken Verbreitungs-Typ („long-distance distribution type“) ersetzt.
Larix decidua wies mit 14 Symbiosepartnern eine hohe Artenvielfalt auf. *Wilcoxina mikolae* und verschiedene Lärchenspezifische *Suillus spp.* (*S. aeruginascens, S. grevillei, S. tridentinus*) wurden in allen Forstgärten nachgewiesen. Ansonsten war die Artenzusammensetzung Forstgarten-spezifisch. Die Wurzelspitzen von *Picea abies* waren nur mit drei Pilzarten assoziiert: *Wilcoxina* sp., *Tuber puberulum*, *Amphinema byssoides*. Die zwei untersuchten Forstgärten unterschieden sich hinsichtlich der Artenvielfalt oder Abundanzen der Symbiosepartner nicht signifikant voneinander. *Pinus cembra* Pflanzen wiesen ebenfalls eine hohe Diversität an Symbiosepartnern auf (12). Die Diversität war in den untersuchten Forstgärten ähnlich, aber die Artenzusammensetzung der Symbiosepartner war teilweise verschieden. *Wilcoxina* sp. und *Suillus spp.* (*S. placidus, S. plorans, S. sibiricus*) waren in allen Forstgärten dominant.
Obwohl die Pflanzen teilweise im selben Forstgärten gezogen worden waren, unterschieden sich die Symbiosepartner der drei untersuchten Pflanzenarten (*Larix decidua*, *Picea abies* und *Pinus cembra*) signifikant in ihrem Artenreichtum und in ihrer Artenzusammensetzung. Letztere war für jede Pflanze spezifisch: *Amphinema byssoides* und *Wilcoxina* sp. 1 waren die einzigen Symbiosepartner, welche auf zwei Wirtspflanzen nachgewiesen werden konnten, auf *Picea abies* und auf *Pinus cembra*. Die Pilz-Gesellschaften von *Larix decidua* und *Pinus cembra* wurden durch wirtsspezifische Pilzarten charakterisiert (*Suillus spp.*).
Obwohl viele europäische Waldbäume in Symbiose mit Ektomykorrhizapilzen (EM) leben, ist relativ wenig über die Zusammensetzung und Abundanzen der Pilzpartner von Forstgartenpflanzen bekannt. In diesem Projekt geht es um den Mykorrhizierungsstatus von Sämlingen und Setzlingen aus Südtirols Forstgärten. Jede Pflanze bildet mit Pilzen eine Symbiose, welche als Mykorrhiza bezeichnet wird. Die Autoren haben diesen Status im Zeitraum von einem Jahr bei Lärchen-, Fichten- und Zirbenpflanzen untersucht und deren Pilzpartner bestimmt. Dieses Projekt wurde vom Amt für Forstverwaltung Bozen gefördert. Betreuerin und Mitautorin dieser Projektarbeit ist Frau A. Univ. Prof. Dr. Ursula Peinter.

Schlüsserwörter: Ektomycorrhiza, Forstgarten, *Suillus*, *Wilcoxina*, Morphotyp, PCR

Riassunto

La maggioranza dei nostri alberi vivono in simbiosi con funghi, le ectomicorrhize (EM). Però poco è noto sulla composizione e le abbondanze delle associate specie funghine. Per questo motivo abbiamo investigato i funghi associati a *Larix decidua*, *Picea abies* e *Pinus cembra*, cresciuti in sei vivai forestali dell'Alto Adige (Aicha/Aica, Prad/Prato, Prettau/Predoi, Radein/Redagno, Ulten/Ultimo, Welsberg/Monguelfo). Lo scopo di principale era l' identificazione dei partner funghini dominanti, e il confronto delle tre specie di piante. Oltre, eravamo interessati a sapere se ci fossero delle differenze in merito alla ricchezza delle EM-specie e della EM-composizione tra provenienze di semi diverse, tra vivai forestali, e riguardando alla stagione o all'età delle piante.

I campioni sono state presi in autunno 2006 e in primavera 2007. Le radici micorrhizate furono divise in morphotipi (MT), e sequenze rDNA ITS furono generate per alcune radici rappresentative di ogni MT. Sono state analizzate 28.290 radici, identificando in sequito i funghi associati a 39 MT. La micorrhizazione era generalmente alta, 100 % in autunno, e 97 % in primavera. La micorrhizazione più bassa in primavera è possibilmente riconducibile al forte impulso di crescita delle piante durante questo periodo.

La composizione delle specie funghine associate alle tre piante investigate si distingueva in modo significativo. Riguardante *L. decidua* e *Pinus cembra* si può anche parlare di comunità funghine specifiche per ogni vivaio. La provenienza di semi delle piante forestale non era un fattore significante per la composizione dei funghi nel vivaio. Ma l'età delle piante oppure la stagione influenzavano la composizione delle specie funghine associate: il complesso *Wilcoxina mikoale* era dominante sulle piantine più giovani, ma veniva presto sostituito dai funghi specifici per la specie di pianta, oppure da funghi con un meccanismo di distribuzione a lunga distanza ("long distance distribution type").

La diversità delle specie fughine associate a *Larix decidua* erano alta (14 spp.). *Wilcoxina mikolae* e fungi specifici per il larice (*S. aeruginascens, S. grevillei, S. tridentinus*) sono stati osservati in tutti e tre vivai forestali. Altrimenti, la composizione delle specie può essere riguardata come specifica per ogni vivaio forestale.*Picea abies* piante erano associate a tre specie di funghi: *Wilcoxina* sp., *Tuber puberulum*, *Amphinema byssoides*. Non cerano differenze significanti tra i due vivai forestali analizzati a proposito della composizione delle specie funghine e della quantità delle specie. Anche *Pinus cembra* piante erano associate ad un alto numero dei funghi (12 *spp*.). La diversità fungina era più o meno uguale negli vivai analizzati, ma la composizione delle specie funghie symbiontiche era in parte differente. *Wilcoxina* sp. e *Suillus spp*. (*S. placidus, S. plorans, S. sibiricus*) erano dominante nei tutti i vivai forestali.

Le tre specie di piante si distinguevano riguardo alla ricchezza delle e alla composizione delle specie funghine associate, nonostante che siano cresciute (almeno in parte) negli stessi vivai forestali. La composizione delle comunità funghine micorrhiziche era specifica per ogni pianta: *Amphinema byssoides* e *Wilcoxina* sp. 1 erano le uniche specie associate a due piante, *Picea abies* e *Pinus cembra.* Le comunità funghine associate a *L. decidua* e *Pinus cembra* era caratterizzate dalla presenza dei funghi specifici (*Suillus spp*.).

Parole chiave: ectomicorrhiza, vivaio forestale, *Suillus*, *Wilcoxina*, morphotipo, PCR

Introduction

Most European forest trees live in symbiosis with ectomycorrhizal (EM) fungi (Iwanski 2006). EM fungi facilitate both nutrient and water uptake, increase resistance to certain root diseases, and enhance the stress tolerance of the tree (Harley & Smith 1983; Allen 1991). Protection from soil-borne pathogens, especially in the early stage of tree development, is also considered as an important function of mycorrhizal symbiosis (Duchesne 1994).

Forest nurseries produce plant material for afforestation. Nursery plants are usually mycorrhized at a high degree (Rudawska 2006), thus bringing their mycorrhizal symbionts from the nurseries to the field. Well developed mycorrhizas may improve the growth and survival of out planted seedlings in the field (Kropp & Langlois 1990), and it has been suggested, that forest tree seedlings with multiple mycorrhizas can withstand a wider range of planting site conditions than plants with only one species of EM fungi (Le Tacon et al 1986, Marx 1982). Most studies on EM community structure have examined mature forests (Dahlberg et al 1997, Dickie & Reich 2005, Erland et al 1999, Gardes & Bruns 1996, Peter et al 2001), or disturbed habitats (Jansen & Dighton 1990, Kåren et al 1997, Lilleskov et al 2002, Peter et al 2001, Stendell et all 1999), whereas relatively little is known about the species composition and abundances of nursery plant mycobionts: some investigations were carried out in forest nurseries in European Nordic countries, most of them focussing on *Picea* and *Pinus spp.* (Iwanski et al 2006, Jonson et al 1999, El Karkouri 2005, Menkis et al 2005, Rudawska et al 2006, Tedersoo et al 2008, Trocha et al 2006), and one recent study also focussed on saprobial fungi colonizing decayed roots of *Pinus sylvestris* and *Picea abies* seedlings (Menkins et al 2006). But nursery mycobionts of *Larix decidua* and *Pinus cembra* were never investigated systematically. These plants are important for the alpine environment, and often used for afforestation at the timberline. Moreover, there is an increasing demand for these timber types for construction purposes in Central, Eastern and Northern Europe because of its static strength, natural durability and its appearance.

The aim of this study was to monitor the EM symbiosis partner of *Larix decidua*, *Picea abies* and *Pinus cembra* seedlings and plantlets in South Tyrolean nurseries. We were especially interested in the following questions: What are the dominating fungal mycobionts? Are there differences in EM species richness and composition between seedling provenances of one plant species, between different plant species, between nurseries and between seasons?

Material und methods

Nursery and seedlings

The sampling of the different plants was performed in several seedling nurseries in South Tyrol: *Larix decidua* seedlings were taken in the nurseries Welsberg/Monguelfo, Prad/Prato and Aicha/Aica; *Picea abies* seedlings were sampled in Ulten/Ultimo and Welsberg/Monguelfo, and *Pinus cembra* in Prettau/Predoi, Radein/Redagno and Ulten/Ultimo (**Table 1**).

Samples were taken in autumn 2006 and spring 2007; autumn sampling was performed on at least three different spots of the nursery and spring samples were taken close to autumn`s sampling spots. Plantlets were classified according their age and seedling origin: a 3+1 means that plants were grown in a seedbed for 3 years and then transferred to transplant beds, where they grew for 1 year; S1 plants were grown in a seedbed for 1 year.

Table 1: List of the analysed plantlets (*Larix decidua* = LÄ, *Picea abies* = FI, *Pinus cembra* = ZI) from South Tyrolean seedling nurseries. The age in years, the classification, the nursery code and the origin of each seedling are given. Asterisks indicate plants which were not available for spring sampling.

	Years	Classification	Code	Origin
Prettau/ Predoi	4	3+1	ZI98a	St. Martin in Thurn/San Martino in Badia
	6	3+3	ZI89a	Ahrntal/Valle Aurina
Radein/Redagno	1	S1	ZI103	St. Martin in Thurn/San Martino in Badia
	1	S1	ZI104	St. Martin in Thurn/San Martino in Badia
	2	S2	ZI101	Langtaufers/Vallelunga
	2	S2	ZI102	Würzjoch/Passo delle Erbe
	3	S3	ZI100	Würzjoch/Passo delle Erbe
	4	S4	ZI98	Langtaufers/Vallelunga
Ulten/Ultimo	3	2+1	ZI100	Stilfs/Stelvio
	5	3+2	ZI96	Graun in Vinschgau/Curon Venosta
	6	4+2	ZI91	St. Martin in Thurn/San Martino in Badia
	1	S1	FI70	Stilfs/Stelvio
	2	S2	FI68	Stilfs/Stelvio
	4	2+2	FI61	Welschnofen/Nova levante
	4	2+2	FI60	Stilfs/Stelvio
Welsberg/Monguelfo	1	S1	FI72	Ratschings/ Racines
	1	S1	FI73	Prags/Valle di Braies
	1	S1	FI74	Welschnofen/Nova levante
	2	S2	FI66	Ratschings/Racines
	2	S2	FI65	Prags/Valle di Braies
	2	S2	FI67	Welschnofen/Nova levante
	4	2+2	FI57	Pragser See/Lago di Braies
	4	2+2	FI58	Ratschings/Racines
	5	2+3	FI50	Welschnofen/Nova levante
	5	2+3	FI55	Prags/Valle di Braies
	5	2+3	FI53	Ratschings/Racines
	3	1+2	LÄ053A*	Südtirol/Alto Adige
	3	2+1	LÄ051A	Ahrntal/Valle Aurina
	3	2+1	LÄ052A	Zentralalpenhauptkamm
	3	2+1	LÄ055B*	Martell/Martello
	4	2+2	LÄ046A	Alpenhauptkamm

	Years	Classification	Code	Origin
Prad/Prato	1	S1	LÄ062A	Alpenhauptkamm-Samenplantage
	1	S1	LÄ063A	Alpenhauptkamm-Samenplantage
	2	S2	LÄ060A*	Ahrntal/Valle Aurina
	2	S2	LÄ058A*	Martell/Martello
	2	S2	LÄ059A*	Alpenhautkamm-Samenplantage
	3	1+2	LÄ055A*	Martell/Martello
	3	1+2	LÄ054A*	Taufers im Münstertal/Tubre
	3	1+2	LÄ044A*	Taufers im Münstertal/Tubre
Aicha/ Aica	1	S1	LÄ061T*	Aicha/Aica
	2	S2	LÄ056T*	Aicha/Aica

Sample processing

All together, about 500 seedlings were investigated, at least 30 plants from each classification. Plants were sampled with abundant adjacent soil. The root systems were gently washed in tap water over a 2 mm sieve to remove most of the soil and organic debris, minimizing damage to the ectomycorrhizas. Material, which was adhering tightly on the root system, was removed with forceps. For randomisation, ectomycorrhized root systems were distributed in a Petri dish of 14 cm and then specific number of mycorrhized and non-mycorrhized fine roots was randomly taken. The same number of root tips was analysed for each plant species and nursery: 600 root tips were investigated for each classification of *L. decidua*, 450 for *P. abies*, and 600 for *P. cembra*. The number of analysed root tips per plant varied from 30 to 300, depending on the number of available plants. All analysed root tips were examined with a Nikon SMZ800 stereomicroscope at 10- to 100- fold magnification. Ectomycorrhiza morphotypes were defined based on colour, emanating elements, mantle layer and hyphal anatomy. From each morphotype at least 4 root tips were stored in Eppendorf-tubes containing 50 µl cetyltrimethyl ammonium bromide (CTAB) buffer at -20 °C until further processing.

Molecular identifications of ectomycorrhizas

Up to 4 root tips per ectomycorrhiza morphotype were analysed for each classification and nursery. DNA was extracted from individual root tips following Southworth (2000; http://www.sou.edu/BIOLOGY/Faculty/Southworth/CTAB.htm). Single root tips were ground in a 1.5 ml Eppendorf tube containing 50 µl CTAB buffer. After adding 550 µl CTAB buffer (final concentration per sample: 12.5 mg hexadecyltrimethylammoniumbromide, 10 mM Tris-HCl (pH 8), 1.4 M NaCl, 20 mM EDTA, 0.2 % β-mercaptoethanol) the samples were incubated at 65 °C for 40-60 minutes, then centrifuged for 7 minutes at 16000 g. The supernatant was precipitated using an equal volume of chloroform. After centrifugation for 15 minutes at 16,000 g, the upper phase was transferred into a new Eppendorf tube containing 750 µl cold (-20 °C) 98 % isopropyl alcohol, and then stored in the freezer (-20 °C) from 30 minutes to overnight for precipitation. After centrifugation for 30 minutes at 16000 g the pellet was washed with 200 µl cold (-20 °C) 70 % alcohol, followed by centrifugation for 5 minutes at 7000 g. Supernatant was decanted, and this washing step was repeated a second time. Then, the uncapped Eppendorf tubes were invert until dry, and dried DNA pellets were resuspended in 50 µl distilled water.

Five microliters of diluted DNA extracts were combined with 20 µl PCR mix, containing 10× buffer S (10 mM Tris-HCl, 50 mM KCL, 1.5 mM MgCl2), 5× enhancer (10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA and 50 % glycerine), deoxyribonucleotide triphosphate, peqGOLD Taq DNA polymerase (all Peqlab, Erlangen, Germany) and primers. The final concentration of these components in 25 µl reactions mix were: 200 µM of each of 2′- deoxyadenosine 5′ triphosphate, 1.25 U of Taq polymerase, 0.4 mM of each primer, 0.64 mM Tris–HCl, 53.2 mM KCl, 1.5 MgCl2, 6.4 µM EDTA and 0.8 µl glycerine.
PCR was performed using a Primus 96 advanced thermocycler (Peqlab) with the following conditions: an initial step of 5 minutes at 94 °C was followed by 40 cycles of denaturation at 94 °C for 1 minute, annealing at 50 °C for 55 seconds (annealing time increasing 3 seconds each cycle) and extension at 72 °C for 45 seconds. Thermal cycling was ended by a final extension at 72 °C for 6 minutes.
The following primer combinations were used: ITS1F×LR15, ITS1F×LR21, ITS1F×NL4 and ITS1F×ITS4. Purified PCR products (ExoSAP-IT PCR Clean- up Kit, GE-Healtcare Europe GmbH, Austria) were sent to Genoscreen (Lille, France) for sequence analyses with the primer ITS1.
Due to the high diversity of ectomycorrhizal morphtoypes from the trees investigated, only the most common MT were analysed.

Data analyses

Resulting rDNA ITS sequences were edited and checked using Sequencher (version 4.6; Gene codes Inc. Ann Arbor, MI). Blast searches were carried out against the public sequence databases National Centre of Biotechnology Information (NCBI) and UNITE (Kõljalg et al. 2005). Sequences with at least 97 % similarity were defined as one Operational Taxonomic Unit (OTU) and regarded as belonging to one species.
Statistical analyses were carried out with relative abundance of morphotypes. Thus, we regarded morphotypes as synonymous for OTUs in these analyses.
The results were tested with the statistical program SPSS (SPSS 15., © 2007 for Windows, SPSS Inc., Chicago USA).

Results

Larix decidua

Larix mycobionts in South Tyrolean nurseries

Larix plants were generally 100 % mycorrhized. All together, 14 fungal mycobiont species were detected on the one to three year old plants. Species richness increased with plant age, and species composition changed significantly from seedlings to plantlets: *Wilcoxina mikolae* was the most important mycobiont of larch seedlings, while *Suillus spp*. was found to be the most important partner of larch plantlets. Species composition varied considerably between nurseries, only *Wilcoxina mikolae* and several *Suillus spp*. occurred in all three nurseries: *Wilcoxina mikolae* was especially abundant on seedlings (S1), being later replaced by other mycobionts, especially *Suillus spp*. (**Table 2**). Plantlets of the nurseries Welsberg/Monguelfo and Prad/Prato had a similarly species richness, but the species composition was different: *Larix*-specific fungi (*Suillus luteus* and *S. viscidus*) were observed in both forest nurseries, otherwise there were no overlapping mycobiont species. A comparison with pot plants (nursery Aicha/Aica) is difficult, but *Suillus spp.* also occurred on these plants.

Table 2: Ectomycorrhizal partner of *Larix decidua* (LÄ) in the forest nurseries Welsberg/Monguelfo, Prad/Prato and Aicha/Aica.

Mycobiont species	Forest nursery					Genbank accession number	Best blast match/ voucher ID	Score	Similarity	Sequence length
	Welsberg/Monguelfo		Prad/Prato		Aicha/Aica					
	A	S	A	S	A					
Amanita aff.		WLÄ053A				GU181845	FJ596775	295	95 %	441
Amanita arctica					ALÄ060A	GU181837	UDB002308	1,045	Locked	634
Cortinarius vernus	WLÄ046A					GU181852	FJ039539	1,469	99 %	877
Exidiopsis sp.	WLÄ053A					GU181854	AY509549	569	89 %	617
Hebeloma bruchetii	WLÄ051A					GU181844	AY948195	1,356	99 %	708
			PLÄ044A			GU181869	AY948195	1,211	98 %	647
Hebeloma incarnatulum		WLÄ046A				GU181850	AF430291	1,556	96 %	952
Hygrophorus speciosus			PLÄ058A			GU181855	DQ097884	345	90 %	651
Melinomyces bicolor	WLÄ051A					GU181856	EF093183	976	95 %	599
Pyronemataceae		WLÄ046A				GU181851	FJ013060	1,441	97 %	808
		WLÄ051A				GU181861	FJ013060	1,053	97 %	602
	WLÄ051A					GU181862	EF644144	1,324	99 %	773
Scleroderma areolatum	WLÄ052A					GU181838	EU819438	1,34	99 %	785
Sowerbyella radiculata					ALÄ056A	GU181839	UDB000985	555	95 %	791
Suillus aeruginascens					ALÄ061A	GU181868	UDB000985	684	97 %	874
			PLÄ055A				AJ272400	1,17	97 %	723
Suillus grevillei					ALÄ056A	GU181857	M91614	1,128	99 %	733
"Suillus luteus"		WLÄ052A				GU181843	DQ367918	1,279	95 %	791
		WLÄ046A					DQ367918	1,152	96 %	985
			PLÄ062A				DQ367918	1,235	95 %	766
				PLÄ062A		GU181848	DQ367918	1,273	95 %	877
Suillus tridentinus		WLÄ046A				GU181860	IB20070485	944	99 %	492
		WLÄ052A				GU181849	IB20070485	1144	99 %	899
Tomentella bryophila		WLÄ052A				GU181846	UDB000254	1,144	99 %	899
		WLÄ051A				GU181859	UDB000254	533	99 %	273
Wilcoxina mikolae 1	WLÄ053A					GU181863	DQ06900	963	99 %	779
		WLÄ052A				GU181871	DQ06900	745	96 %	428
			PLÄ053A			GU181864	DQ06900	961	99 %	789
			PLÄ062A			GU181841	DQ06900	955	99 %	616
			PLÄ054A			GU181866	DQ06900	944	99 %	495
				PLÄ062A		GU181840	DQ06900	1,045	100 %	881
				PLÄ063A		GU181842	DQ06900	1,047	99 %	794
					ALÄ056A	GU181870	DQ06900	866	100 %	411
					ALÄ063A	GU181858	DQ06900	1,015	99 %	810
Wilcoxina mikolae 2			PLÄ062A			GU181853	AJ893249	991	93 %	793
Wilcoxina mikolae 3		WLÄ052A				GU181847	DQ093774	908	99 %	482

Nursery Welsberg/Monguelfo

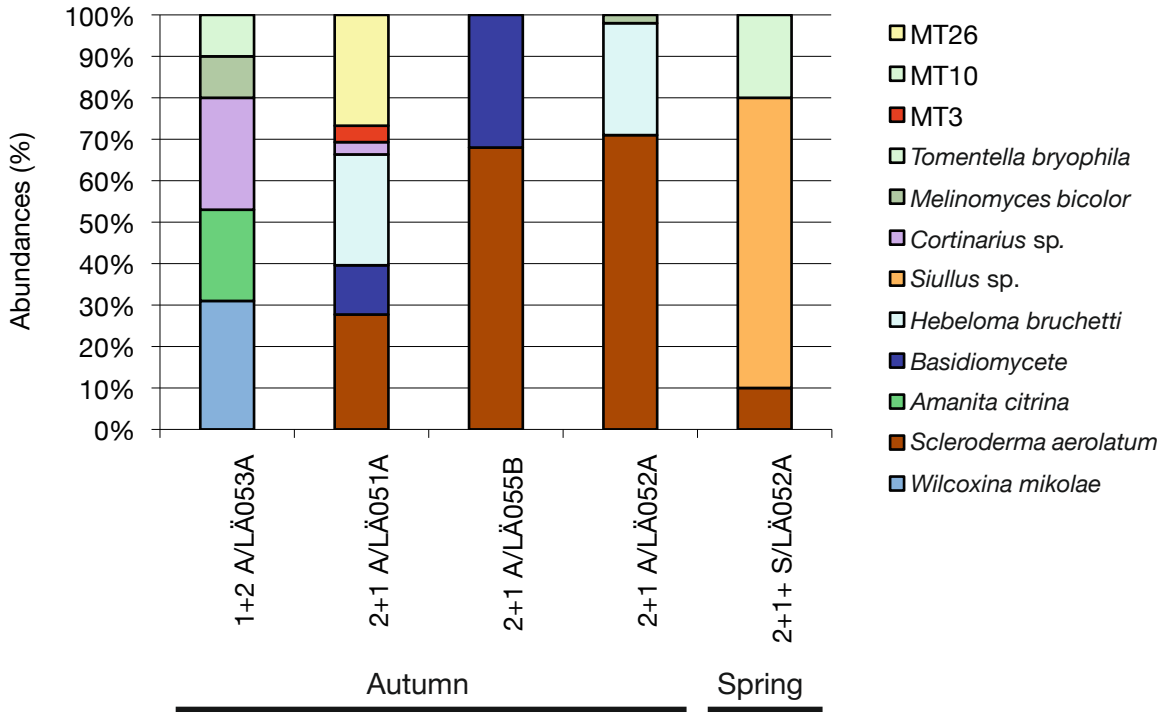
Larix decidua root tips were 100 % ectomycorrhized. All together, ten mycobionts were identified from autumn samples (October 23, 2006), 2-4 species occurring on each provenance. *Scleroderma areolatum* occurred on plants from three provenances and was the overall dominant symbiosis partner in this nursery (42 % abundances, **Table 3**). No significant differences were found between provenances.

In spring (July 10, 2007), three mycobiont species were detected: *Scleroderma areolatum* was found again on the same plant classes, all other mycobionts were replaced by *Suillus luteus, S. tridentinus* and *Tomentella bryophila* (**Fig. 1**). Due to this replacement, species assemblage and the abundances were significantly different between autumn and spring ($p = 0.004$).

Table 3: Relative ectomycorrhizal abundances (percent) of *L. decidua* plants from the nursery in Welsberg / Monguelfo in autumn (A) 2006 and spring (S) 2007. Morphotype number (MT), the number of analysed plants, species richness and rate of mycorrhization (n = 600 root tips analysed) are given.

Season	Code	Years	Classification	*Amanita citrina*	Basidiomycete	*Cortinarius* sp.	*Hebeloma bruchetii*	*Melinomyces bicolor*	*Scleroderma areolatum*	*Suillus spp.*	*Tomentella bryophila*	*Wilcoxina mikolae*	MT3	MT10	MT26	Analysed plants	Species richness	Rate of mycorrhization
Autumn	1+2 A/LÄ053A	3	1+2	22		27		10				31		10		5	6	100
	2+1 A/LÄ051A	3	2+1		12	3	27		28				4		27	2	6	100
	2+1 A/LÄ052A	3	2+1				27	2	71							2	4	100
	2+1 A/LÄ055B	3	2+1		32				68							2	2	100
Spring	2+1+ S/LÄ052A	3	2+1+						10	70	20					6	3	100

Fig. 1: Relative ectomycorrhizal abundances (percent) of fungal partner of *L. decidua* from the seedling nursery in Welsberg/Monguelfo. *Scleroderma areolatum* was the most common mycorrhizal partner in autumn, in spring all mycobiont species were replaced by *Suillus spp.* and *Tomentella bryophila*.



Nursery Prad/Prato

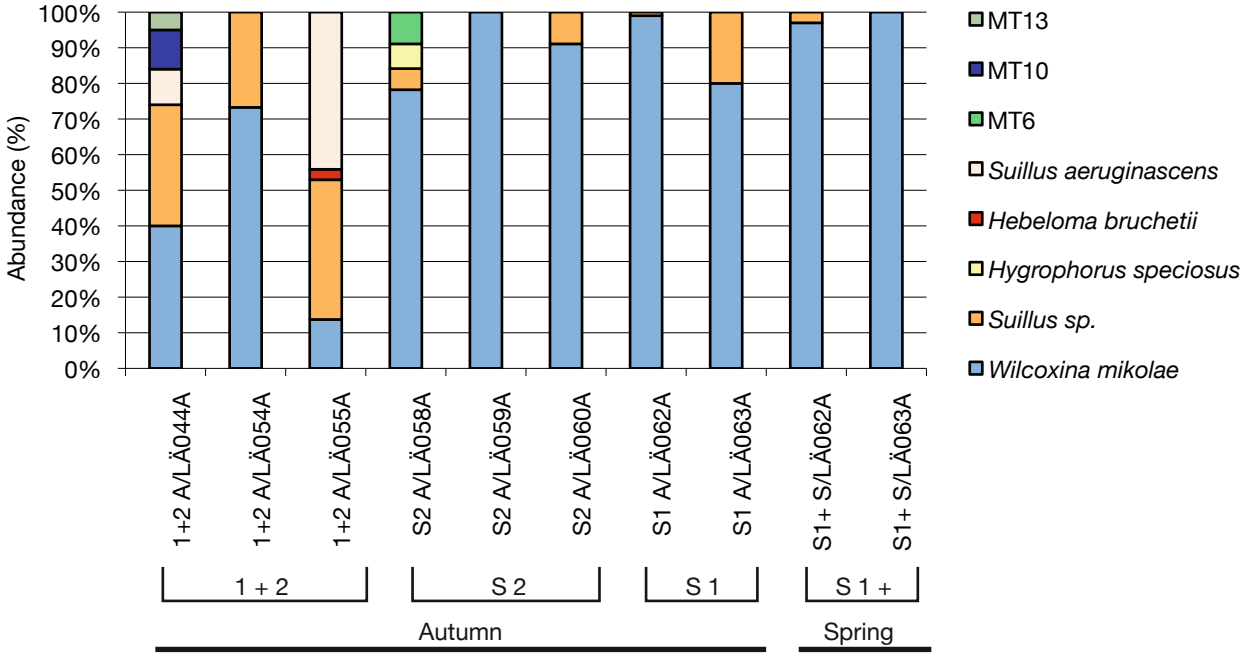
Root tips were always 100% mycorrhized. Eight fungal partner were observed in autumn, (October 25, 2006), *Wilcoxina mikolae* 2 being the most abundant species (72% abundances) (**Table 4**). Other important species were *Suillus luteus* and *S. aeruginascens* (17%). *Wilcoxina mikolae* was generally the most abundant mycorrhizal partner, colonizing more than 97% of the root tips of one- and two year old seedlings. Older plants have a higher mycobiont diversity, with especially *Suillus spp.* (24%) abundances increasing.

Three fungal partner were observed in spring (June 02, 2007): same as in autumn, *Wilcoxina mikolae* and *Suillus luteus* were detected on one year old seedlings (S1) (**Fig. 2**). Older plants were not available for sampling in spring.

Table 4: Relative ectomycorrhizal abundance (percent) of *L. decidua* plants from the nursery in Prad/Prato in autumn (A) 2006 and spring (S) 2007. Morphotype number (MT), the number of analysed plants, species richness and rate of mycorrhization (n = 600 root tips analysed) are given.

Season	Code	Years	Classification	*Hebeloma bruchetii*	*Hygrophorus speciosus*	*Suillus aeruginascens*	*Suillus luteus*	*Wilcoxina mikolae* 1	*Wilcoxina mikolae* 2	MT6	MT10	MT13	Plants analysed	Species richness	Rate of mycorrhization
Autumn	S1 A/062A	1	S1				1	99					12	2	100
	S1 A/063A	1	S1				20	80					12	2	100
	S2 A/060A	2	S2				9	92					6	2	100
	S2 A/058A	2	S2		7		6	79		9			6	4	100
	S2 A/059A	2	S2					101					6	1	100
	1+2 A/055A	3	1+2	3		45	40	14					5	4	100
	1+2 A/054A	3	1+2				27	74					5	2	100
	1+2 A/044A	3	1+2			10	34	40			11	5	5	5	100
Spring	S1+ S/062A	1+	S1+				3		97				12	2	100
	S1+ S/063A	1+	S1+					100					12	1	100

Fig. 2: Abundances (in percent) of the 8 fungal partner of *L. decidua* from the nursery in Prad/Prato. *Wilcoxina mikolae* 1+2 was the most common mycorrhizal partner on one and two year old seedlings. Three years old plants showed an increase in mycorrhizal abundances of *Suillus spp*.



Nursery Aicha/Aica

Pot plants from the nursery in Aicha/Aica, were sampled only in autumn (November 07, 2006). The roots were 100 % mycorrhized. Five fungal partner were observed: *Sowerbyella radiculata* (36 %) and *Suillus tridentinus* (21 %) were the most abundant species (**Table 5**). *Suillus luteus*, *Wilcoxina mikolae* 1 and 2 *Amanita arctica* were also identified. *Suillus tridentinus* was found only on two-year old plants, *Suillus luteus* was present on one and two year old plants (**Fig. 3**).

Table 5: Relative ectomycorrhizal abundances (percent) of *L. decidua* plants from the nursery in Aicha/Aica in autumn (A) 2006 and spring (S) 2007. Morphotype number (MT), the number of analysed plants, species richness and rate of mycorrhization (n = 600 root tips analysed) are given.

Season	Code	Years	Classification	*Amanita arctica*	*Suillus luteus*	*Suillus tridentinus*	*Sowerbyella radiculata*	*Wilcoxina mikolae* 1	Plants analysed	Species richness	Rate of mycorrhization
Autumn	S1 A/LÄ061T	1	S1		16		70	15	10	3	100
	S2 A/LÄ056T	2	S2	24	16	42	2	16	5	5	100

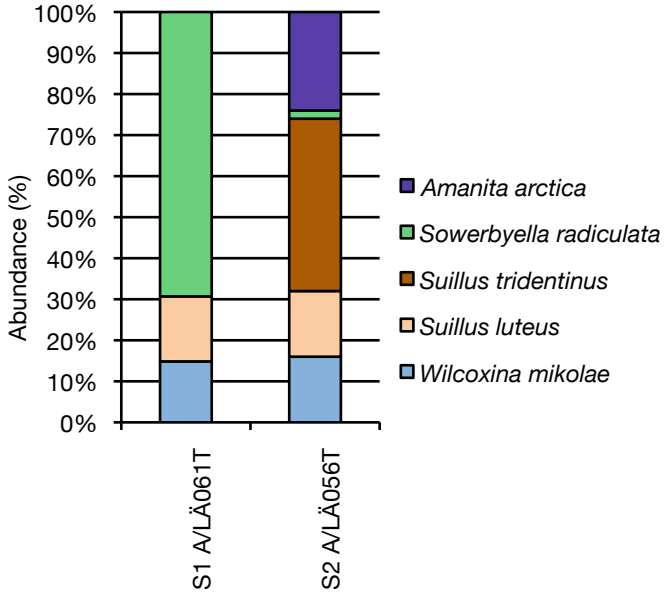


Fig. 3: Abundances (in percent) of *L. decidua* mycobionts from the nursery in Aicha/Aica. *Sowerbyella radiculata* was the most common mycorrhizal partner on one year old seedlings whereas *Suillus tridentinus* was most frequent on two year old plants.

Fig. 4: Ectomycorrhizal morphotypes of Larix decidua from the seedling nurseries in South Tyrol/Alto Adige: 1 = *Suillus cavipes*; 2 = *Lactarius* sp.; 3, 5 and 6: *Tomentella bryophila*; 4 = *Suillus tridentinus*. Bar: 1 - 4 = 1 mm; 5 - 6 = 0.5 µm

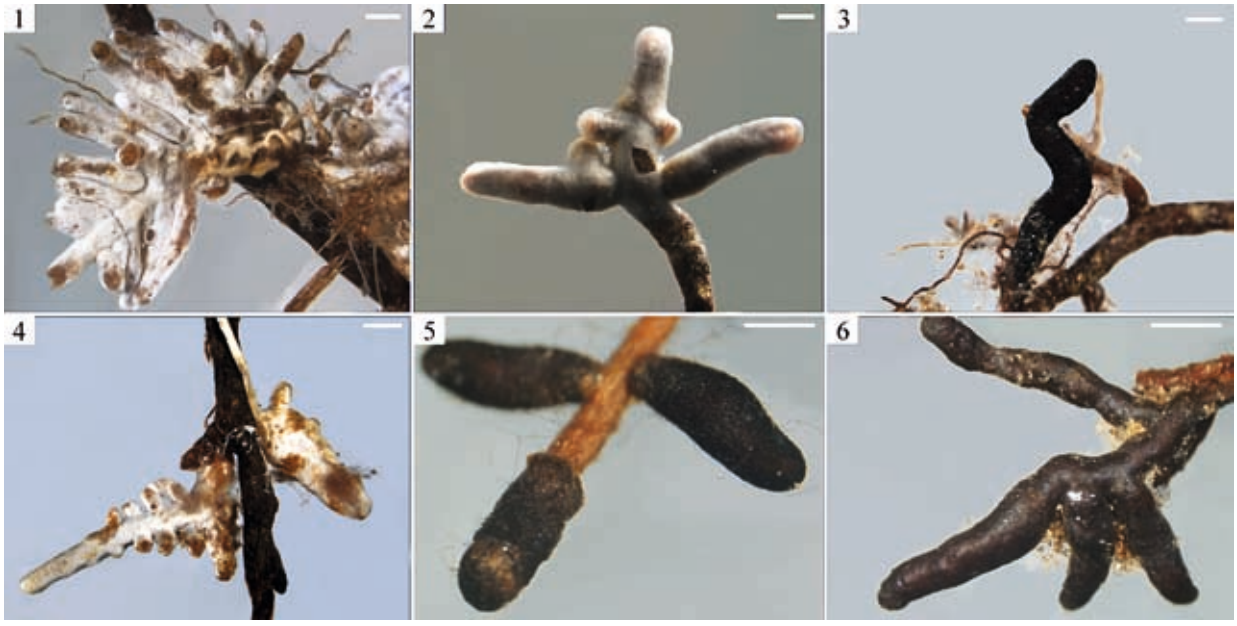


Fig. 5: Nursery plants of *L. decidua*, Welsberg/Monguelfo 23.10.2006

Fig. 6: Nursery plants of *L. decidua*, Prad/Prato 25.10.2006

Picea abies

Picea abies mycobionts in South Tyrolean nurseries

Root tips of *Picea abies* had always high mycorrhization rates (93-100%), but species richness was generally low (3 fungal species) (**Table 6**). Regarding mycobiont species composition and relative abundances, the two analysed nurseries did not differ from each other. Species composition changed with plant age, *Wilcoxina* sp. 4 being replaced by *Amphinema byssoides*. Seedling provenances were not found to be significant for mycobiont species composition in these nurseries.

Table 6: Ectomycorrhizal partner of *Picea abies* (FI) in the nurseries Ulten/Ultimo and Welsberg/Monguelfo in autumn 2006 and spring 2007.

Species	Forest nursery				Genbank accession number	Best blast match / voucher ID	Score	Similarity	Sequence length
	Ulten/Ultimo		Welsberg/ Monguelfo						
	Autumn	Spring	Autumn	Spring					
Amphinema byssoides	FI60				GU181872	AY838271	1604	98 %	873
		FI68			GU181873	AY838271	1243	97 %	708
				FI50	GU181874	AY838271	906	98 %	850
Tuber puberulum	FI70				GU181875	AY751559	1043	95 %	807
		FI70				UDB000122	1116	99 %	713
		FI68			GU181877	AJ969625	1128	95 %	854
			FI74		GU181876	AJ969615	1116	98 %	854
				FI66	GU181878	DQ069050	930	99 %	489
Wilcoxina sp. 4	FI68				GU181879	DQ508797	926	100 %	494
		FI70			GU181880	DQ069052	862	98 %	786
			FI66		GU181881	DQ069052	890	98 %	819
				FI66	GU181882	DQ069052	866	98 %	810

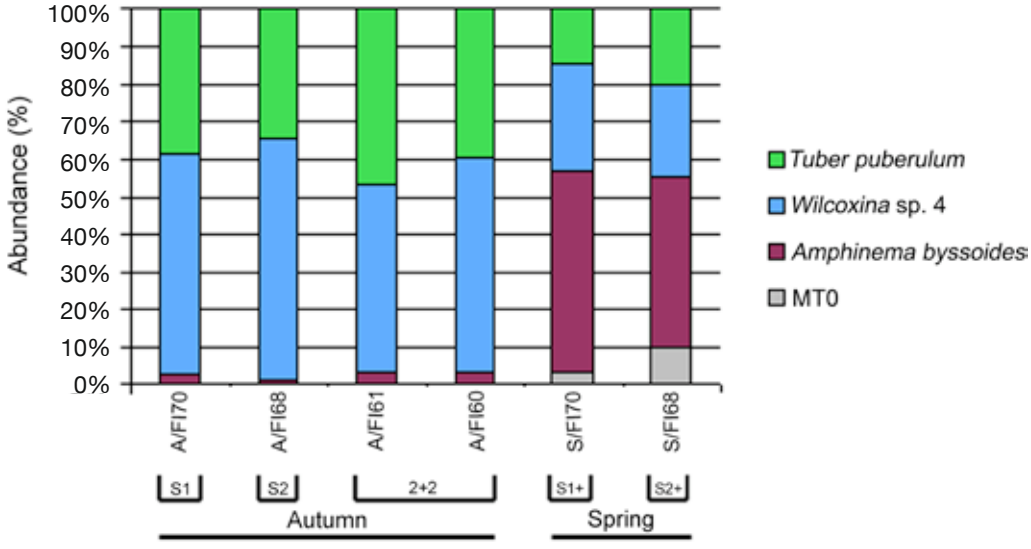
Nursery Ulten/Ultimo

Picea abies nursery plant root tips were 100% mycorrhized in autumn (November 07, 2006) and 93% in spring (April 03, 2007). Three mycobionts occurred on all provenances and during both seasons: *Wilcoxina* sp. 4 (57%) and *Tuber puberulum* (40%) were the dominant mycorrhizal partner, *Amphinema byssoides* (3%) was an additional fungal partner observed in autumn 2006 (**Table 7**). Abundance of *A. byssoides* increased (49%) in spring, with *Wilcoxina* sp. 4 (27%) and *Tuber puberulum* (17%) decreasing. Fungal species composition did not differ significantly between seasons of provenances (**Fig. 7**).

Table 7: Relative ectomycorrhizal abundances (percent) of *Picea abies* plants from the nursery in Welsberg / Monguelfo in autumn (A) 2006 and spring (S) 2007. Morphotype number (MT), the number of analysed plants, species richness and rate of mycorrhization (n = 450 (A); 240 (S) root tips analysed) are given. MT0 are unmycorrhized root tips.

Season	Code	Years	Classification	*Amphinema byssoides*	*Tuber puberulum*	*Wilcoxina* sp. 4	MT0	Analysed plants	Species richness	Rate of mycorrhization
Autumn	S1 A/FI70	1	S1	12	174	264	0	15	3	100
	S2 A/FI68	2	S2	6	155	289	0	15	3	100
	2+2 A/FI61	4	2+2	9	113	118	0	8	4	100
	2+2 A/FI60	4	2+2	9	94	137	0	8	4	100
Spring	S1+ S/FI70	2	S1+	240	65	130	15	15	3	96
	S2+ S/FI68	3	S2+	205	90	110	45	15	3	90

Fig. 7: Abundances (in percent) of the 3 established fungal partner of *Picea abies* from the nursery in Ulten / Ultimo in autumn (A) 2006 and spring (S) 2007. *Amphinema byssoides* increased in spring. MT0 are unmycorrhized root tips.



Nursery Welsberg/Monguelfo

Picea abies nursery plant root tips were 100% mycorrhized in autumn (October 23, 2006) and 94% mycorrhized in spring (May 29, 2007). Three mycobionts occurred during both seasons, *Wilcoxina* sp. 4, *Tuber puberulum* and *Amphinema byssoides*: Two fungal partner occurred on seedlings (S1 and S2), three on plantlets (2+2 and 2+3) (**Table 8**). *Wilcoxina* sp. 4 and *Tuber puberulum* were the most dominant mycorrhizal partner during both seasons, with abundances of 41% / 39% and 42 / 34% (**Fig. 8**). *Amphinema byssoides* was absent on S1 seedlings, but became dominant on older plantlets. No significant differences were found between season and provenances.

Fig. 8: Abundances (in percent) of the 3 established fungal partner of *Picea abies* from the seedling nursery in Welsberg/Monguelfo in autumn (A) 2006 and spring (S) 2007. *Amphinema byssoides* was absent on S1 seedlings, but became dominant on older plantlets and in spring. MT0 are unmycorrhized root tips.

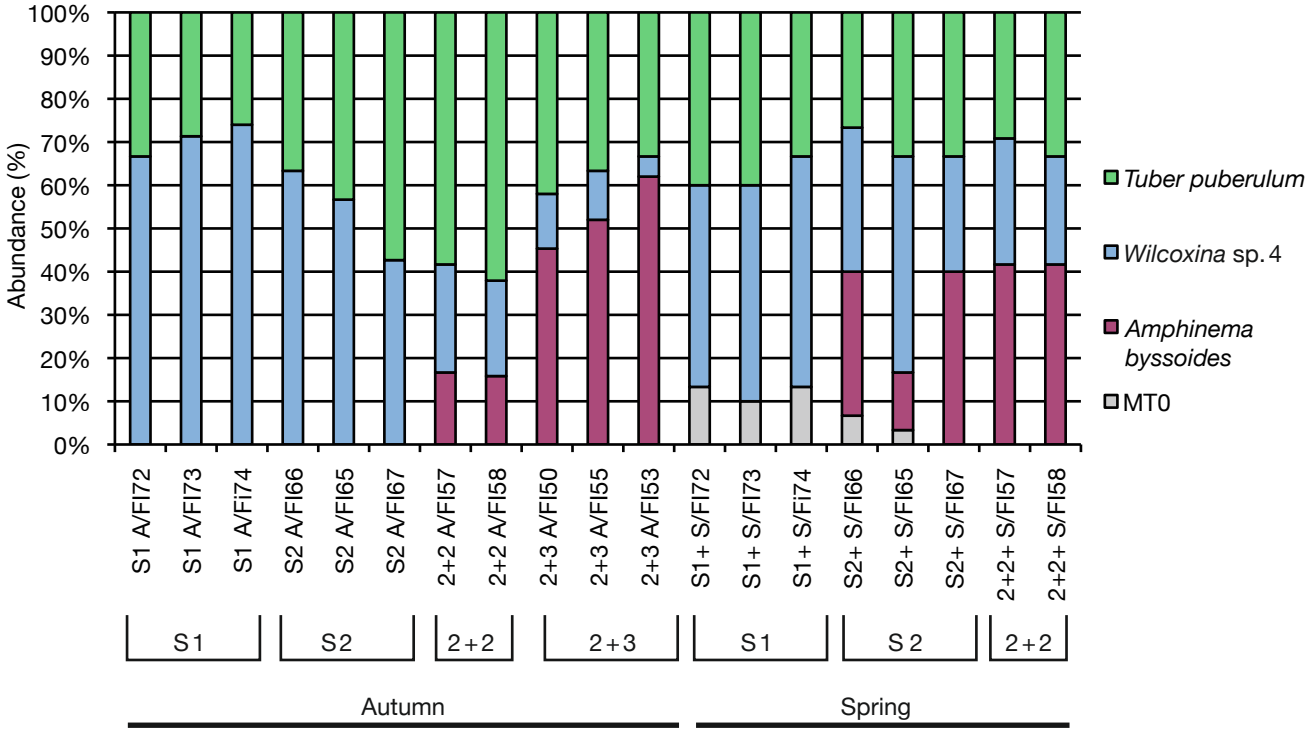


Table 8: Relative ectomycorrhizal abundances (percent) of *Picea abies* plants from the nursery Welsberg/ Monguelfo in autumn (A) 2006 and spring (S) 2007. Morphotype number (MT), the number of analysed plants, species richness and rate of mycorrhization (n = 150 root tips analysed, except A/FI57 and A/FI58: n = 240) are given. MT0 are unmycorrhized root tips

Season	Code	Years	Classification	*Amphinema byssoides*	*Tuber puberulum*	*Wilcoxina* sp. 4	MT0	Analysed plants	Species richness	Rate of mycorrhization
Autumn	S1 A/FI72	1	S1	0	50	100	0	5	2	100
	S1 A/FI73	1	S1	0	43	107	0	5	2	100
	S1 A/Fi74	1	S1	0	39	111	0	5	2	100
	S2 A/FI66	2	S2	0	55	95	0	5	2	100
	S2 A/FI65	2	S2	0	65	85	0	5	2	100
	S2 A/FI67	2	S2	0	86	64	0	5	2	100
	2+2 A/FI57	4	2+2	40	140	60	0	8	3	100
	2+2 A/FI58	4	2+2	38	149	53	0	8	3	100
	2+3 A/FI50	5	2+3	68	63	19	0	5	3	100
	2+3 A/FI55	5	2+3	78	55	17	0	5	3	100
	2+3 A/FI53	5	2+3	93	50	7	0	5	3	100
Spring	S1+ S/FI72	2	S1+	0	60	70	20	5	2	92
	S1+ S/FI73	2	S1+	0	60	75	15	5	2	96
	S1+ S/Fi74	2	S1+	0	50	80	20	5	2	92
	S2+ S/FI66	3	S2+	50	40	50	10	5	2	90
	S2+ S/FI65	3	S2+	20	50	75	5	5	2	96
	S2+ S/FI67	3	S2+	60	50	40	0	5	2	86
	2+2+ S/FI57	5	2+2+	100	70	70	0	8	3	96
	2+2+ S/FI58	5	2+2+	100	80	60	0	8	3	91

Fig. 9: Ectomycorrhizal morphotypes of *Picea abies* from the seedling nurseries in South Tyrol/Alto Adige: 1 and 2: *Wilcoxina spp;* 3 = *Amphinema byssoides*. Bar: 50 µm

Fig. 10: Nursery plants of *Picea abies* , Ulten/Ultimo 07.11.2006

Fig. 11: Nursery plants of *Picea abies* , Welsberg/Monguelfo 23.10.2006

Pinus cembra

Pinus cembra mycobionts in South Tyrolean nurseries

Root tips of *Pinus cembra* showed always high mycorrhization rates (93-100 %), and a comparatively high (12) species richness (**Table 9**). No statistically significant differences could be found regarding season and provenances. Nurseries did not differ significantly from each other in mycobiont species richness, but species composition was partly different: *Wilcoxina spp*. dominated on young plants in all nurseries; besides that, *Suillus plorans* was dominating in Prettau/Predoi, *Cortinarius flexipes* and *S. plorans* in Ulten/Ultimo, and *Inocybe rimosa* and *S. sibiricus* in Radein. *Wilcoxina spp.*, *Suillus spp.* and *Tomentella sp.* were the only *Pinus cembra* mycobionts occurring in all three nurseries.

Table 9: Ectomycorrhizal partner of *Pinus cembra* (ZI) in the forest nurseries Prettau/Predoi and Radein/Redagno in autumn 2006 and spring 2007.

Species	Forest nursery						Genbank accession number	Best blast match/ voucher ID	Score	Similarity	Sequence length
	Prettau/Predoi		Radein/Redagno		Ulten/Ultimo						
	Autumn	Spring	Autumn	Spring	Autumn	Spring					
Amphinema byssoides	ZI98a						GU181883	AY838271	660	95 %	432
		ZI098a					GU181884	AY838271	904	98 %	871
Cortinarius flexipes	ZI98a							AJ889971	609	96 %	471
					ZI100		GU181885	AJ889971	701	97 %	482
					ZI96			AJ889971	632	98 %	798
						ZI91		AJ889971	529	99 %	692
Inocybe dulcamara		ZIS1						AM882765	722	97 %	869
Inocybe rimosa			ZI98				GU181886	AJ8889957	632	96 %	712
Melinomyces bicolor	ZI098a						GU181887	EF093183	878	95 %	600
			ZI103				GU181888	AY394885	977	96 %	600
Sebacina sp.	ZI98a						GU181889	DQ974767	666	95 %	579
Suillus placidus	ZI89a						GU181890	AB28443	961	99 %	550
Suillus plorans	ZI98a						GU181891	AY272417	1185	99 %	790
		ZIS1					GU181893	AY272417	944	98 %	693
		ZI98a					GU181894	AY272417	1185	99 %	820
					ZI91		GU181892		987	97 %	778
						ZI91			918	98 %	398
Suillus sibiricus			ZI104				GU181895	UDB000690	906	99 %	469
			ZI100				GU181897	AF166512	862	96 %	565
			ZI98				GU181899	AF166512	1160	98 %	684
				ZI104			GU181896	AF166512	1092	98 %	676
				ZI102			GU181898	AF166512	1154	98 %	842
Tomentella sp.	ZI98a						GU181900	AJ534912	1354	99 %	732
					ZI91			AJ534912	1103	99 %	
Wilcoxina mikolae			ZI102				GU181901	DQ069052	930	99 %	796
Wilcoxina sp. 4		ZI98a					GU181902	DQ508810	938	100 %	476
				ZI102			GU181904	DQ069052	317	93 %	647
	ZI98a							DQ069052	920	99 %	760
						ZI100	GU181903	DQ069052	782	99 %	429
					ZI91			DQ069052	628	99 %	648

Nursery Prettau/Predoi

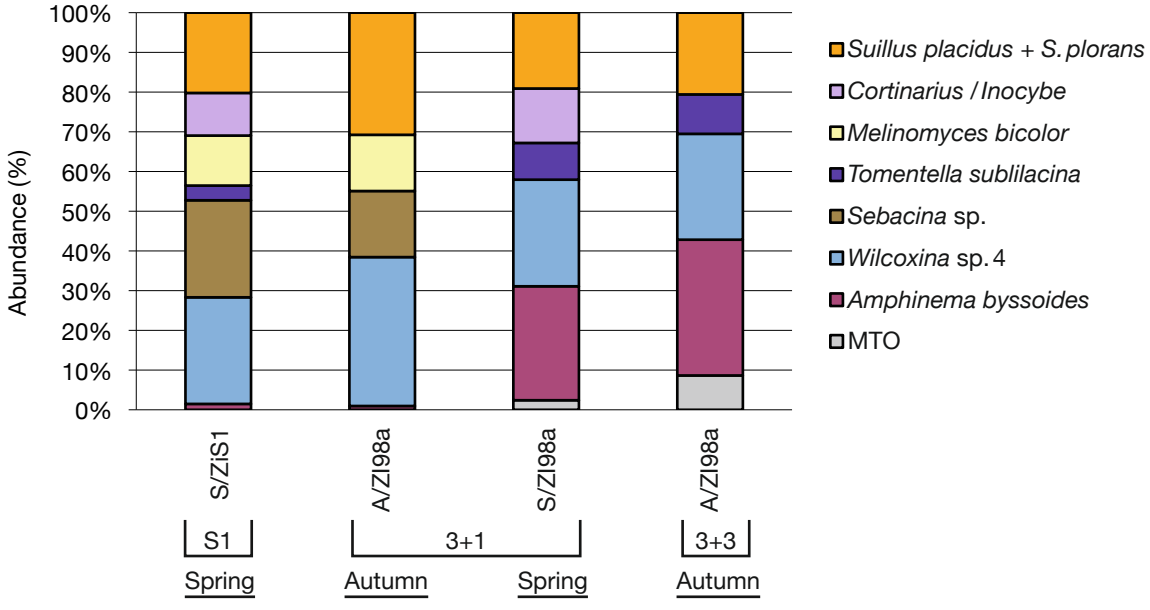
Pinus cembra root tips were 100% mycorrhized. Seven, respectively five mycobiont species were detected on the root tips of the 4- and 6-year old seedlings in autumn (October 17, 2006); four mycobiont species occurred on the 5-year old plants in the following spring (April 03, 2007), five species occurred on the 1-year old seedlings (**Table 10**). *Wilcoxina* sp. 4 (28% / 33%) and *Suillus plorans* (32% / 23%) were the dominating mycorrhizal partner. *Amphinema byssoides*, *Cortinarius flexipes*, *Inocybe dulcamara*, *Tomentella* sp., *Melinomyces bicolor*, *Suillus placidus* and *Sebacina* sp. were also found. *Amphinema byssoides* occurred with higher abundances in spring, replacing *Melinomyces bicolor* and *Sebacina* sp. (**Fig. 12**).

Species richness decreased with increasing age of *Pinus cembra* plants, and the number of mycobiont partner was higher in autumn than in spring.

Table 10: Relative ectomycorrhizal abundances (percent) of *Pinus cembra* mycobionts from the nursery Prettau/Predoi in autumn (A) 2006 and spring (S) 2007. Morphotype number (MT), the number of analysed plants, species richness and rate of mycorrhization (n = 600 root tips analysed) are also given. MT0 are unmycorrhized root tips.

Season	Code	Plant age	Classification	*Amphinema byssoides*	*Cortinarius / Inocybe*	*Melinomyces bicolor*	*Sebacina sp.*	*S. placidus + S. plorans*	*Tomentella* sp.	*Wilcoxina* sp. 4	MT0	Analysed plants	Species richness	Rate of mycorrhization
Spring	S1 S/ZiS1	1	S1	155	74	0	0	163	50	145	13	20	5	95
Autumn	3+1 A/ZI98a	4	3+1	8	58	68	132	169	20	145	0	20	7	100
Spring	3+1+ S/ZI98a	5	3+1+	242	0	0	0	112	54	145	47	20	4	91
Autumn	3+3 A/ZI89a	6	3+3	5	0	74	87	238	0	196	0	20	5	100

Fig. 12: Abundances (in percent) of *Pinus cembra* mycobionts from the nursery in Prettau/Predoi in autumn (A) 2006 and spring (S) 2007. *Wilcoxina* sp. 4 and *Suillus placidus* were the most dominant mycobionts. MT0 are unmycorrhized root tips.



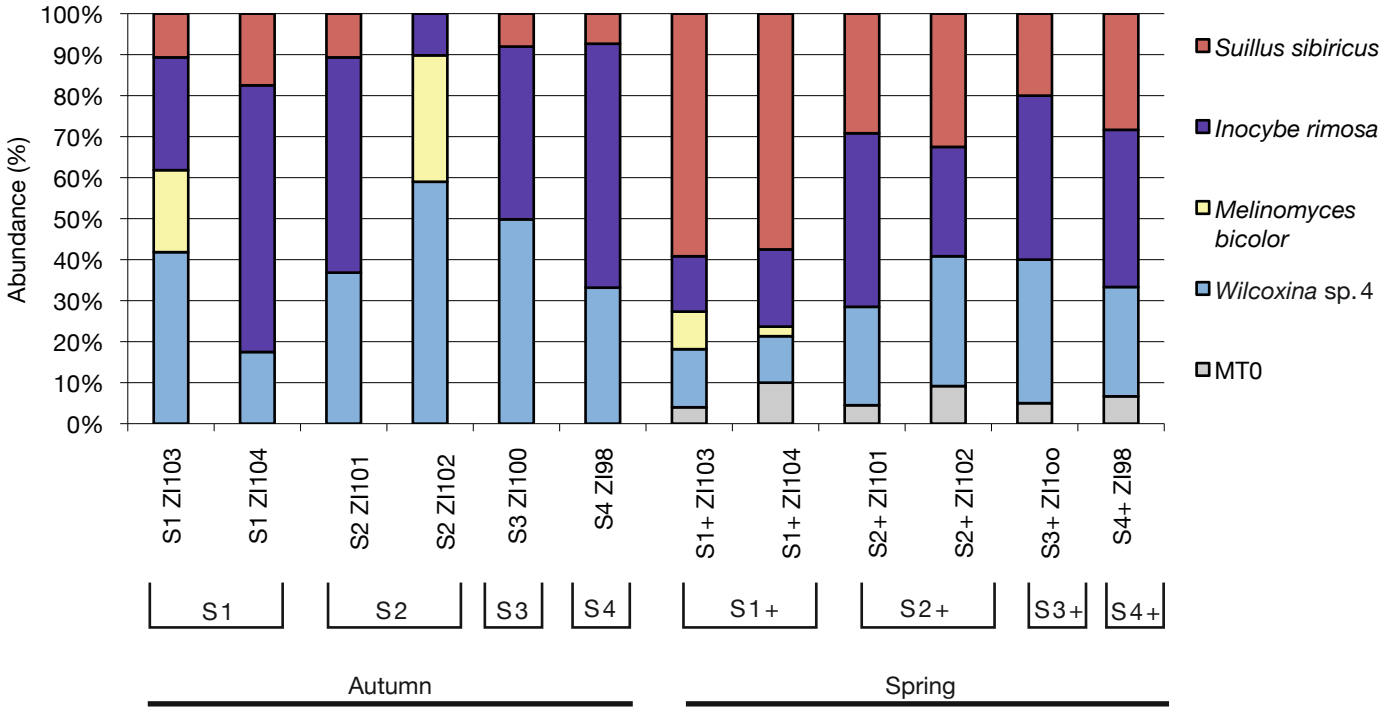
Nursery Radein/Redagno

Pinus cembra root tips were mycorrhized at 100% in autumn (October 2006), and at 93% in spring (June 2007). Four mycorrhizal partner were found on the 2-, 3-, 4- and 5-years old plants (**Table 11**). *Wilcoxina* spp. (40% / 24%) and *Inocybe rimosa* (43% / 30%) were the most dominant fungal partner. Additionally, *Suillus sibiricus* and *Melinomyces bicolor* were found, *S. sibiricus* being more abundant in spring, and *Melinomyces bicolor* more abundant on younger (1-2 year old) plants (**Fig. 13**). Regarding season, provenances and classification there were no significant differences.

Table 11: Relative ectomycorrhizal abundances (percent) of *Pinus cembra* mycobionts from the seedling nursery in Radein/Redagno in autumn (A) 2006 and spring (S) 2007. Morphotype number (MT), the number of analysed plants, species richness and rate of mycorrhization (n = 600 root tips analysed) are given. MT0 = unmycorrhized root tips.

Season	Code	Years	Classification	*Inocybe rimosa*	*Melinomyces bicolor*	*Suillus sibiricus*	*Wilcoxina* sp. 4	MT0	Analysed plants	Species richness	Rate of mycorrhization
Autumn	S1 A/ZI103	1	S1	165	120	64	251	0	20	5	100
	S1 A/ZI104	1	S1	387	0	104	104	0	20	5	100
	S2 A/ZI101	2	S2	315	0	64	221	0	20	3	100
	S2 A/ZI102	2	S2	61	185	0	354	0	20	3	100
	S3 A/ZI100	3	S3	252	0	48	298	0	20	3	100
	S4 A/ZI98	4	S4	357	0	44	199	0	20	3	100
Spring	S1+ S/ZI103	2	S2	81	55	355	85	24	20	5	93
	S1+ S/ZI104	2	S2	113	14	345	68	60	20	5	93
	S2+ S/ZI101	3	S3	254	0	175	144	27	20	3	95
	S2+ S/ZI102	3	S3	160	0	195	190	55	20	3	96
	S3+ S/ZI100	4	S4	240	0	120	210	30	20	3	97
	S4+ S/ZI98	5	S5	230	0	170	160	40	20	3	97

Fig. 13: Abundances (in percent) of *Pinus cembra* mycobionts from the nursery in Radein/Redagno in autumn (A) 2006 and spring (S) 2007. *Wilcoxina* sp. 4 and *Inocybe rimosa* were the most dominant mycorrhizal partner. *Suillus sibiricus* became more abundant in spring. MT0 are unmycorrhized root tips.



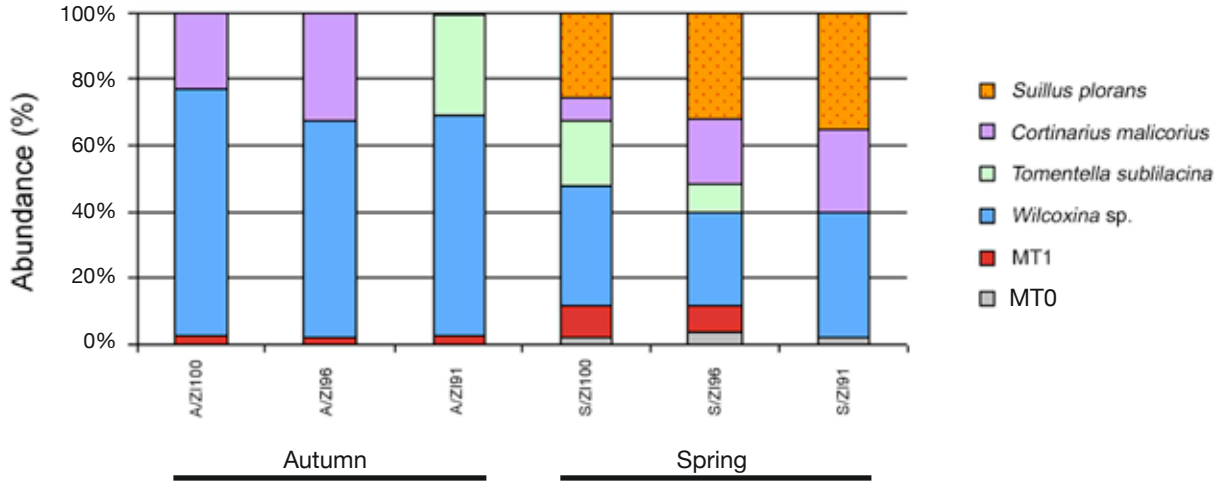
Nursery Ulten/Ultimo

Pinus cembra root tips from the forest nursery in Ulten/Ultimo were 100% mycorrhized in autumn (25 October 2006), and 98% in spring (June 02, 2007). In total, five fungal partner were found on the 3-7 years old plants (**Table 12**). *Wilcoxina* sp. 4 (69%) and *Cortinarius felxipes* (18%) dominated in autumn; abundances of *Wilcoxina* sp. 4 (34%) decreased in spring, while *Suillus plorans* (30%) abundances increased (**Fig. 14**). *Amphinema byssoides* (2%) and *Tomentella sp*. (9%) occurred with low abundances.

Table 12: Relative ectomycorrhizal abundances (percent) of *Pinus cembra* mycobionts from the nursery Ulten/Ultimo in autumn (A) 2006 and spring (S) 2007. Morphotype number (MT), the number of analysed plants, species richness and rate of mycorrhization (n = 600 root tips analysed, except autumn A/ZI100 and A/ZI96 n = 300) are given. MT0 are unmycorrhized root tips.

Season	Code	Years	Classification	*Cortinarius malicorius*	*Suillus plorans*	*Tomentella* sublilacina	*Wilcoxina* sp. 4	MT0	MT1	Analysed plants	Species richness	Rate of mycorrhization
Autumn	2+1 A/ZI100	3	2+1	68	0	0	225	0	7	10	3	100
	3+2 A/ZI96	5	3+2	97	0	0	198	0	5	10	3	100
	4+2 A/ZI91	6	4+2	0	2	182	395	0	16	20	4	100
Spring	2+1+ S/ZI100	4	2+2	45	150	120	215	10	60	20	5	89
	3+2+ S/ZI96	6	3+3	120	190	50	170	20	50	20	5	98
	4+2+ S/ZI91	7	4+3	150	210	0	230	10	0	20	3	94

Fig. 14: Abundances (in percent) of *P. cembra* mycobionts from the nursery Ulten/Ultimo in autumn (A) 2006 and spring (S) 2007. *Wilcoxina* sp. 4 and *Cortinarius malocorius* were the most dominant mycorrhizal partner. *Suillus plorans* became more abundant in spring. MT0 are unmycorrhized root tips.



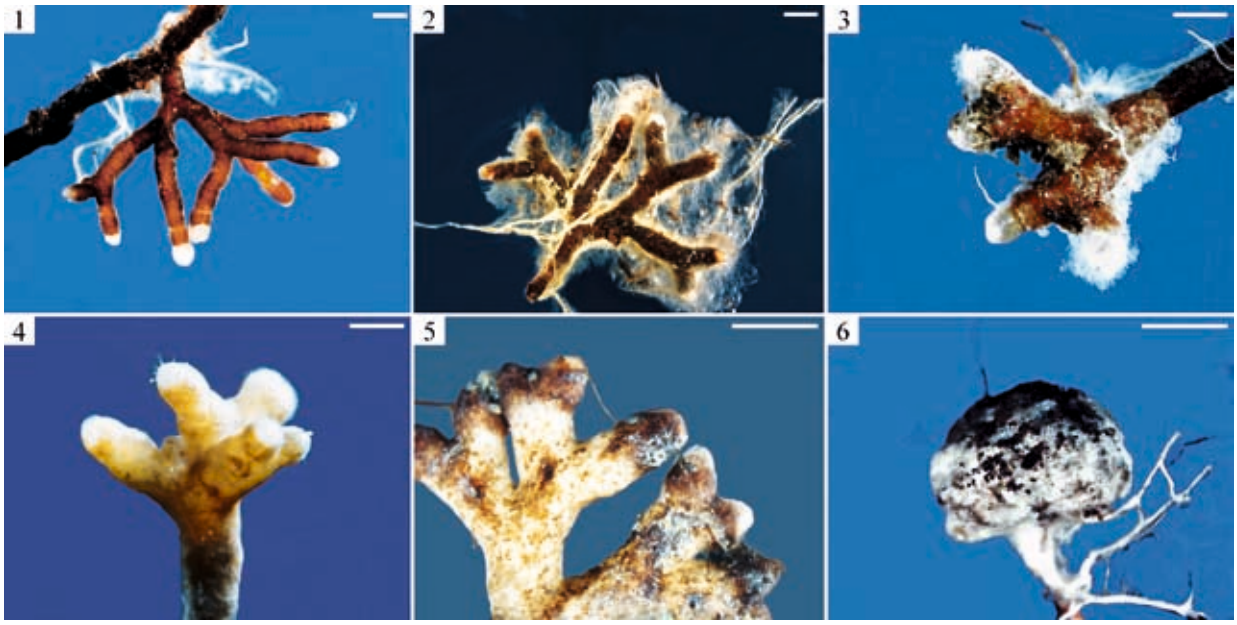


Fig. 15: Ectomycorrhizal morphotypes of *Pinus cembra* from the seedling nurseries in South Tyrol/Alto Adige:

1 = *Wilcoxina* sp.4;
2 = *Amphinema byssoides*;
3 = *Cortinarius flexipes*;
4 = *Melinomyces bicolor*;
5 = *Suillus sibiricus*;
6 = *Suillus plorans*. Bar: 50 µm

Fig. 16: Nursery plants of *Pinus cembra*, Prettau/Predoi 15.10.2006.

Discussion

The mycorrhiza status of *Larix decidua* nursery plants in South Tyrol

Fungal symbiosis partner are important for development of larch plantlets, as shown by the constant degree of 100 % mycorrhization. The total mycobiont species richness (14; 3-8 /nursery) of *Larix decidua* plants in South Tyrolean nurseries was comparatively high. Interestingly, mycobiont species composition varied considerable between the three nurseries and also between seasons. Thus, we have to consider the *Larix*-associated fungal species composition as specific for each nursery. However, some mycobionts appear to be especially important for development of larch plantlets: *Wilcoxina mikolae* and larch-specific *Suillus spp*. occurred in all three nurseries: *Wilcoxina mikolae* was especially abundant on seedlings, being later replaced by other mycobionts, especially *Suillus spp*..
Comparison of our results with literature data is difficult, as very little is known about the mycorrhiza status of *L. decidua* in Europe: an early investigation by Göbl (1974) showed that inoculation with *Larix*-specific mycobionts (*Suillus grevillei*) resulted in a higher shoot length, an increased number of side shoots, and a higher spear and root dry weight than in not inoculated control plants.
Based on the fact, that *Larix*-specific *Suillus spp.* were abundantly found in all South Tyrolean nurseries, we consider these nursery plants as well prepared for fast and good development after outplanting. Further, field studies with outplanted material are needed for proving that mycorrhizal symbioses established in the nursery have a lasting effect.

The mycorrhiza status of *Picea abies* nursery plants in South Tyrol

Mycorrhization degree was constantly high in *Picea abies* nursery plants, but mycobiont species richness was low (3 species): *Wilcoxina* sp., *Tuber puberulum* and *Amphinema byssoides* occurred in all nurseries with similar abundances and dynamics, *A. byssoides* abundances increasing with plant age. Our results are in concordance with investigations on the mycorrhization status of *Picea abies* plantlets from other European forest nurseries: they also detected a high mycorrhization degree in combination with low species richness: Baier et al. (2006) found only one mycorrhizal partner on the root tips of *P. abies* in forest nurseries; Menkis et al. (2005) analysed *P. abies* seedlings from Latvian forest nurseries and found similar mycobionts communities, *Wilcoxina sp.*, *Thelephora terrestris*, *Phialophora finlandii* and *Tuber* sp. dominating. Moreover, they report that species richness of mycorrhizal partner of *P. abies* was significantly lower than species richness of *Pinus* nursery plants. Rudawska (2006) extensively analysed the mycorrhization of *P. abies* in 17 Polish forest nurseries. She detected a total of 12 mycobiont species, with an average of 3.7 mycobionts per nursery on 1-4 years old *P. abies* plantlets. Besides *Wilcoxina mikolae, Amphinema byssoides* and *Tuber* sp., she also detected other common mycobionts like *Thelephora terrestris*, *Cenococcum geophilum* and *Phialophora finlandia*. As remarkable accordance with our study, *Wilcoxina mikolae* was the dominant partner of 1-2 years old *Picea* plantlets. Summarizing all available studies of *Picea* plantlets in European forest nurseries, *Wilcoxina* sp., *Amphinema byssoides* and *Tuber* sp. can be considered as typical mycorrhizal community of 1-4 years old *Picea abies* plantlets.
Wilcoxina is generally a very common fungal partner of seedlings in forest nurseries (Danielson 1991, Egger 1995, Rudawska 2006). This ascomycete follows a ruderal life strategy, and forms resistant resting structures (chlamydospores), which quickly germinate after disturbance. *Wilcoxina* mycorrhizas usually persist only in the absence of other fungal mycobionts (Danielson & Pruden 1990). This typical early stage mycobiont is not competitive under constant or suitable environmental conditions. The high abundances of *Wilcoxina spp*. show that forest nurseries can be accounted as disturbed habitats (Rudawska 2006), to which fungi following a ruderal life strategy are adapted best. Species delimitation of the *Wilcoxina mikolae* complex is critical: Based on 98 % ITS sequence similarity, four different taxa of *Wilcoxina mikolae* were detected in

South Tyrolean forest nurseries. *Wilcoxina mikolae* 1-3 was found on *Larix decidua* roots only, while another, closely related taxon (*Wilcoxina* sp.) dominated on *Picea* plantlets. This finding indicates host specificity of *Wilcoxina* taxa. Although plants came partly from the same nursery, *Wilcoxina* sp. was found on *Picea abies* and *Pinus cembra* only, and *Wilcoxina mikolae* 1-3 on *Larix decidua* only.
Tuber mycobiont species are also typical for forest nurseries, but with much lower abundances. We speculate that this fungus has either a low competitiveness, or a comparatively low inoculum potential.
Amphinema byssoides is a typical pioneer- and "multi-stage-"ectomycorrhizal partner, which occurs on young and mature plants (Kranabetter 2004). *Amphinema byssoides* builds large networks of mycelium in the soil, which allow a fast new colonization of root tips, but are also susceptible to disturbance. After disturbance this fungus needs longer periods for recovering and rebuilding the mycelial network, maybe one reason for *Amphinema byssoides* occurring mainly on older plantlets.

The mycorrhiza status of *Pinus cembra* nursery plants in South Tyrol

Mycorrhization degree and species richness of *Pinus cembra* mycobionts was generally comparatively high (12; 3-7 *spp.*/nursery). The *P. cembra* mycobiont species composition was nursery-specific, with exception of *Wilcoxina* sp. occurring on seedlings in all nurseries. The constant detection of *Wilcoxina* sp. in South-Tyrolean nurseries is striking, as this mycobiont was not detected in North- and East Tyrolean nurseries: Schmidt (2006) reported only *Tylospora asterophora* in association with one year old *P. cembra* seedlings. She reports a similar species richness (6-10 *spp.*/nursery), but besides *P. cembra*-specific fungi (*Suillus placidus*, *Suillus plorans*, *Suillus sibiricus*) detected on 3-4 years old plants, there was no overlap in species composition with our study.
Suillus mycorrhizas are tubercle-like and form long rhizomorphs, thus belonging to the long distance exploration type as defined by Agerer (2001). Such mycorrhizas are regarded as a very beneficial and efficient for the associated plant. In an early study, Göbl & Heumader (1963) showed that survival rate, growth rate and vitality were much higher in *Pinus cembra* nursery plants associated with host-specific *Suillus spp.* than with unspecialized mycobionts. *Suillus plorans*, *S. placidus* and *S. sibiricus* are host specific multi-stage mycobionts and have a wide geographical distribution (Keller 1996). In the typical subalpine to `Kampfzone` environment of *P. cembra*, these *Suillus spp.* remain the dominating mycobionts over most of the plant lifetime (Heumader & Göbl 1994; Schmidt 2006). Therefore it is certainly a starting advantage for outplanted plants to be equipped with suitable mycobionts from the nursery.

Comparison of different nursery plant species

All investigated plant taxa from South Tyrolean forest nurseries showed a high mycorrhization degree. Although partly being raised in the same nursery, *Larix decidua*, *Picea abies* and *Pinus cembra* differed significantly in mycobiont species richness and species composition: First, species richness of *Picea abies* mycobionts was generally low (max. 3 *spp.*/nursery), compared to *Pinus cembra* (max. 7 *spp.*/nursery) and *Larix decidua* (max. 8 *spp.*/nursery). The mycobiont species richness of *P. cembra* and *Larix decidua* was similar to the species richness of 1-2 years old *Pinus sylvestris* seedlings: Iwanski et al. (2006) found 2-7 mycobiont species per Polish nursery, and reported a total species richness of 12 *spp. Wilcoxina mikolae* and *Thelephora terrestris* dominated on *Pinus* sylvestris seedlings, but also *Suillus*-species were often observed.
Second mycobiont species composition was specific for each plant: *Amphinema byssoides* and *Wilcoxina* sp. 1 were the only mycobiont taxa detected on two plant hosts, *Picea abies* and *Pinus cembra*. The mycorrhizal community of *Larix decidua* and *Pinus cembra* were typically characterised by the occurrence of host specific fungi (*Suillus spp.*). The occurrence of plant specific mycobionts in forest nurseries is considered as important for a faster and better development of the plants, making them less susceptible to biotic and abiotic limiting factors (Göbl & Heumader 1963, Göbl 1974). Seedling provenance was never significant for mycobiont

species composition in nursery plants. However, species composition changed with plant age or season: the *Wilcoxina mikolae* complex dominated on seedlings, being later replaced either by host specific mycobionts, or by mycobionts with a long distance distribution type like *Amphinema* or *Cortinarius*.

Factors, influencing in the mycobiont species composition in nurseries

The lower mycorrhization rate in spring was probably caused by the strong growth impulse of plants during this period. Mycobiont species composition strongly affects the function of mycorrhizal symbiosis in general, because single fungal symbiosis partner differ in their function (Thurner and Pöder 1995). Besides competition and succession, the species composition also depends on many abiotic factors:

Mycorrhizal fungi react sensitive on pH-value, nutrient supply and quality, N-sources, humidity, temperature and radiation (Bidartondo et al. 2001, Smith and Read 1997). As an example, N-fertilization can significantly affect the mycorrhizal species composition: Ammonium is normally the best N-source for fungi, but some species can also use nitrate as single N-source (e.g. *Suillus placidus*, *S. bovinus*, *S. granulatus* and *S. variegatus*). Species unable to catabolising nitrate (e.g. *S. plorans* and *S. sibiricus*) become less competitive due the N-fertilization and are then easily replaced (Keller 1996). The occurrence of *S. plorans* and *S. sibiricus* indicates a sustainable and natural environment-orientated cultivation technique.

Soil cultivation strategies can also influence species composition. Intensive soil cultivation and cultural change negatively affect mycelial networks in the soil, causing a significant reduction of host-specific symbiosis partner and a dominance of ruderal symbiosis partner on seedlings (e.g. *Wilcoxina*).

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