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Testing of microbial additives in the rooting of Norway spruce (*Picea abies* [L.] Karst.) stem cuttings

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ABSTRACT: Laboratory-produced alginate-bead inoculum of ectomycorrhizal (ECM) fungi *Cortinarius* sp. and *Gomphidius glutinosus*, fungal commercial products ECTOVIT® and TRICHOMIL®, bacterial commercial product Bacto-Fil B®, and commercial rooting stimulator VETOZEN® were applied to a peat-perlite (1:2, v:v) rooting substrate of Norway spruce stem cuttings collected from 4-year-old nursery-grown seedlings immediately before the insertion of cuttings into the substrate. The application of beads free of fungi and the substrate without an additive were the other treatments. The cuttings were rooted in vessels (60 cuttings per vessel, 180 per treatment) placed in a glasshouse and arranged in a randomized complete block design. The cuttings were estimated for one growing season (approximately for 26 weeks) after their insertion into the rooting substrate. Rooting % of the cuttings ranged from 45 (mycelium-free beads) to 75 (control) according to treatments, 64 on average. No significant differences among treatments were found in % of ECM morphotypes, total ECM colonization of roots (%), and growth parameters of shoots and roots of the cuttings. The applied microbial additives were not sufficiently efficient to form treatment-related ectomycorrhizas that were formed by naturally occurring ECM fungi. Inoculation by the ECM fungus *Cortinarius* sp. and application of Trichomil had a partial stimulative effect on the shoot growth of cuttings. Shoot and root growth parameters were not significantly correlated with total ECM colonization, except for a negative dependence of the root number in Trichomil treatment. A higher concentration of K but lower concentrations of Ca and Mg in Ectovit treatment than in the other treatments were detected.

Keywords: rooting of cuttings; microbial additives; ectomycorrhizal inoculation; *Picea abies* (L.) Karst.

Vegetative propagation by stem cuttings is a complementary technology of the production of forest tree planting stock in most European countries, used mainly for breeding and reproduction of the gene pool of high-valued populations of forest tree species (Ritchie 1991; Paule 1992). Norway spruce (*Picea abies* [L.] Karst.) is one of the forest tree species with well-handled procedures of vegetative propagation by cuttings. The origin, time and place of cutting collection, age of ortets, size and physiological state of cuttings, rooting substrate and environmental conditions are the traits that significantly affect the success of Norway spruce vegetative propagation (Spethmann 1997; Repáč 2000; Nordborg, Welander 2001; Jurásek, Martincová 2004).

The preparation of cuttings results in a reduction of water and nutrient absorption by cuttings. Consequently, carbohydrates and growth hormones (auxins) produced by aboveground parts of cuttings play an important role in the processes of rhizogenesis and growth of cuttings (Kozlowski, Pallardy 1997; Spethmann 1997). Some growth regulators are excreted (Slankis 1973; Stein, Fortin 1990), and the occurrence of saprophytic and pathogenic microorganisms in rhizosphere is

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restricted by ectomycorrhizal (ECM) fungi (Normand et al. 1996), which can lead to stimulation of rhizogenesis and modification of the root morphology of cuttings even though in the absence of ectomycorrhiza formation (non-specific ECM fungi stimulation) (Chmelíková, Cudlín 1990). ECM symbiosis, a mutualistic relationship between trees and fungi, influences many processes related to physiology, nutrition, growth, and reproduction of forest trees (Kozlowski, Pallardy 1997; Repáč 2000; Courty et al. 2010). In consequence of the modification of root exudates by ECM fungi, specific environment called mycorhizosphere is created in the surroundings of roots. The coexistence of ECM fungi with soil microorganisms, especially with bacteria, facilitates first of all the decomposition of primary soil minerals and thus the plant nutrition (Koele et al. 2009; Courty et al. 2010). Besides that, soil bacteria participate in biogeochemical cycles in rhizosphere and affect the health and growth of trees (Chanway 1997; Hayat et al. 2010).

Chmelíková and Cudlín (1990), Cudlín et al. (1991), Kuděla (1991), Chmelíková et al. (1992) and Repáč (1998, 2000, 2002, 2005, 2006) studied effects of inoculation by ECM fungi on the development of Norway spruce stem cuttings. The results varied from positive to negative inoculation effect. Several authors tested the effect of inoculation by ECM fungi on the development of cuttings of other coniferous trees with similar variable results (Stein et al. 1990; Stein, Fortin 1990; Parladé et al. 1999; Di Battista et al. 2002). Growth hormones indoleacetic acid (IAA), α-naphthalene acetic acid (NAA) and mainly indolebutyric acid (IBA) are the most frequently used rooting stimulators in autovegetative propagation of forest trees by cuttings (Spethmann 1997). The use of other additives potentially supporting the development of cuttings of conifers of the temperate zone is not known in currently available literature.

The objective of this study was to test the effect of laboratory-produced alginate-bead inoculum of ECM fungi *Cortinarius* sp. and *Gomphidius glutinosus* (Schaeff.: Fr.), fungal commercial products ECTOVIT® and TRICHOMIL®, bacterial commercial product BactoFil B®, and commercial rooting stimulator VETOZEN® applied to a rooting substrate on (*i*) rooting, (*ii*) ectomycorrhiza formation, (*iii*) growth of roots and top parts, (*iv*) chemistry of the photosynthetic apparatus of stem cuttings of Norway spruce for one growing season after their insertion into the rooting substrate.

MATERIAL AND METHODS

Cuttings and rooting substrate

The cuttings (7–10 cm long) were collected from the terminal portion of lateral shoots of dormant 1+3 seedlings (certificate number 01564ZV-578 according to Slovak national rules, central Slovakia source) produced in a bed of the forest nursery (altitude 780 m) of Technical University in Zvolen, Slovak Republic, closely before the beginning of growing season (end of April). The cuttings were stored in a refrigerator for a few days and disinfected in a 0.1% solution of the fungicide Fundazol 50 WP for 10 min immediately before they were placed into a rooting substrate (Chmelíková, Cudlín 1990). The cuttings were inserted into the substrate approx. to a 2-cm depth. The rooting substrate, consisting of a mixture of perlite (1 to 4 mm particle size) and peat at a 2:1 (v:v) ratio, was fumigated with the disinfectant Basamid Granular (200 $g \cdot m^{-3}$, 5-day fumigation under a black plastic foil, aeration for additional 14 days before cutting planting). The peat (commercial producer BORA, Ltd., Bobrov, Slovak Republic) was amended with a small portion of perlite, vermiculite, zeolite, bentonite, zeolitic limestone and NPK (14%, 16% and 18% of N, P and K, respectively).

Ectomycorrhizal fungi, commercial products and inoculum preparation

Alginate-bead inoculum of ECM fungi *Cortinarius* sp. (isolate TUZ115) and *Gomphidius glutinosus* (Schaeff.: Fr.) (isolate TUZ500), and commercial products BactoFil B (AGRO.bio Hungary Ltd. Budapest, Hungary), Ectovit (Symbiom, s.r.o., Czech Republic), Trichomil (Jozef Drimal-BIOMO, s.r.o., Slovak Republic) and Vetozen (Geoproduct Healing Mineral Ltd, Mäd, Hungary) were added to the rooting substrate. Addition of pure beads (no fungal mycelium) to the substrate and pure peat-perlite rooting substrate without an additive (control) were the other experimental treatments. Application of the two laboratory-produced inocula + four commercial products + mycelium-free beads + untreated substrate set up 8 treatments of the test effect. BactoFil B contains bacteria which take up N from the air (*Azotobacter*) and vicinity of roots (*Azospirillium*), microorganisms (*Bacillus, Pseudomonas*) transmitting P from soil to plants, growth stimulators, phytohormones, and vitamins. Ectovit contains the mycelium of 4 strains of ECM

fungi, spores of 2 strains of ECM fungi in a peat-based carrier with ingredients supporting the development of ectomycorrhizas (ECMs) (humates, ground minerals, extracts from sea organisms), and naturally degradable granules of a water-retaining gel. Trichomil is a microbial fungicide containing spores of the mycoparasitic fungus *Trichoderma harzianum*, which can control fungal diseases and stimulate plant development via the induction of phytohormone production. Vetozen is a synthetic stimulator intended for the stimulation of rooting of cuttings, germinated seeds or transplanted plants providing an increased content of macro- and micronutrients in rhizosphere (SiO_2, TiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, Na_2O, K_2O).

Mycelial cultures of *Cortinarius* sp. and *Gomphidius glutinosus* were isolated and maintained *in vitro*, and alginate-bead inoculum of these fungi was prepared in a laboratory of the Department of Silviculture, Technical University in Zvolen, Slovak Republic. *Cortinarius* sp. (isolate TUZ115) was isolated on MMN medium in September 1994 (Marx 1969), *Gomphidius glutinosus* (TUZ500) on BAF medium in August 2006 (Moser 1960), both were isolated from sporocarps collected near Zvolen, central Slovakia, and associated with Norway spruce. Sporocarps of the fungi are widely distributed and well-known natural symbionts with Norway spruce in Slovak Republic (e.g. Gáper, Mihál 2008), the mycelium is relatively easy to manipulate in culture. The fungi were maintained as stock cultures alternately on BAF, MMN, KHO (Šašek, Musílek 1967) and malt-peptone (50 ml of brewery wort + 5.0 g of peptone to 1,000 ml of H_2O) (Repáč 2011) media to support the vitality and resistance of cultures.

For the production of fungal inoculum in this experiment a few mycelial discs from the margin of fresh mycelial agar colonies cultured on Petri dishes were removed to 100 ml Erlenmeyer flasks filled with BAF liquid medium and cultivated on a shaker for a few days. Submerged fungal cultures were slightly homogenized, poured into 0.5 or 1.0 l Erlenmeyer flasks filled with BAF liquid medium to a half of the volume and further grown at continual shaking for a few weeks. The mycelium was rinsed with distilled water and homogenized in a blender for a few seconds. The alginate-bead inoculum was prepared according to the procedure of Le Tacon et al. (1983) modified by Kropáček and Cudlín (1989). The principle of the method is immobilization of the fungal mycelium in alginate gel and formation of beads in a calcium chloride solution. The beads were stored in air-tight containers at 4°C and used for the inoculation the next day. The viability of mycelium entrapped in beads was positively tested by cultivation of the beads on agar medium.

Inoculation and rooting of cuttings

Laboratory-produced fungal inoculum and commercial products were applied to the rooting substrate placed in PVC vessels (48 × 15 × 13 cm, length × width × depth) immediately before the insertion of cuttings into the substrate. The beads were mixed with the 4-cm upper layer of rooting substrate at the dose of 250 ml per vessel (3,500 $ml \cdot m^{-2}$) in each of the bead-application treatments. The beads contained approximately 19 and 15 $g \cdot m^{-2}$ of mycelium in dry weight of *Cortinarius* sp. and *Gomphidius glutinosus*, respectively. The product Bactofil B was used in the form of granules (approximately 2 mm in diameter) embedded in a narrow foil strips. The strips were placed in 6 rows (approximately 2 cm apart each other) in each of the concerned vessel approximately 2 cm below the surface of the rooting substrate. Ectovit was applied as slurry (gel) that was prepared by a mixing of the fungal mycelium with dry components of the product (including fungal spores and powder hydrogel) and adequate amount of water. The slurry was thoroughly mixed with the upper half of the volume of rooting substrate in vessels at a ratio 3:5 (v:v). Liquid Trichomil was used as 1% water solution in the dose of 9.0 $l \cdot m^{-2}$ at the placement of cuttings to the substrate and later three times at a month interval starting at the beginning of July. In Vetozen treatment, 1.5 to 2.0 cm basal parts of cuttings were dipped alternately twice into tap water and this powder product.

Three replications (vessels) of 60 cuttings in each treatment (180 cuttings per treatment) were arranged in a randomized complete block design and placed in a glasshouse. The experiment was conducted in the Borová hora Arboretum of Technical University in Zvolen (altitude 330 m, average open air temperature in growing season +15.0°C). The cuttings were rooted in the glasshouse under natural light and temperature regimes. High air humidity was maintained with a room humidifier. The rooting substrate was watered manually if needed. High air temperature in the glasshouse during hot days was decreased by shading and natural aeration. Neither substrate nor air humidity was consistently fully controlled. All cuttings were treated with systemic fungicide several times at the beginning of growing season in order to suppress

diseases caused by pathogenic microorganisms. No further fertilizers or pesticides were applied throughout the experiment.

Sampling, measurements and analyses

After the first growing season (end of October), the cuttings were removed from the substrate and assessed for rooting percentage. Fifteen cuttings from each treatment and block (360 totally) were randomly selected and evaluated for the percentage of ECM colonization, number, total length, average length, dry weight (48 h at 80°C) of roots and shoots, terminal shoot length per cutting, and content of chemical substances in needles. Root systems were gently washed and ECMs were determined under a dissecting microscope at 10–40 × magnification according to gross morphological features such as ramification, shape, colour, outer mantle characteristics, presence of hyphae and rhizomorphs (Möttönen et al. 2001; Repáč 2007). This assessment was made in order to distinguish particular experimental ECM morphological types (morphotypes) and to ascertain whether the inoculation formed a distinctive treatment-related ECM morphotypes. To confirm the formation of ECMs, cross-sections were freehand cut with a razor blade from suspected short roots and examined by light microscopy (400–600 × magnification). The presence of a fungal mantle irrespective of its thickness was considered as an evidence of ECM development. The Hartig net was not always observed on roots with fungal mantle. Based on a preliminary assessment, ECMs were typed to experimental morphotypes according to colour, shape and diameter regardless of the treatment. No further characterization of ECMs was done. Thin, translucent roots lacking a mantle and with root hairs were considered as non-mycorrhizal. The percentage of the number of ECMs of each morphotype from the total number of short roots (all ECMs + non-mycorrhizal roots) was estimated on the root system of each cutting at 10–25 × magnification. The percentage of total ECM colonization was calculated as a sum of percentages of the morphotypes. Chemical analysis of the photosynthetic apparatus was done in a laboratory of National Forestry Centre in Zvolen. Dry matter of needles was determined gravimetrically, total content of C and N by dry combustion, total content of P by colorimetric method, and total contents of K, Ca and Mg by flame atomic absorption spectrometric method.

The experiment was a two-way classification (additive application, block) arranged in a randomized complete block design. The percentages of morphotypes and total ECM colonization and growth parameters were analyzed by analysis of variance followed by Tukey's test to determine differences among treatments. ECM percentages were log transformed prior to statistical analysis. Linear correlation analysis was done to determine the dependence of growth parameters on the percentage of total ECM colonization. All analyses were processed using the PC SAS statistical package.

RESULTS

Rooting % of Norway spruce cuttings rooted in the substrate inoculated with alginate-bead inoculum of ECM fungi or various commercial products ranged from 45 to 75 (Fig. 1), 64 on average. The high rooting % were assessed in cuttings rooted in the uninoculated (control) substrate (75%) and with the application of rooting stimulator Vetozen (71%), the low ones in cuttings inoculated with *G. glutinosus* (56%) and with the application of pure

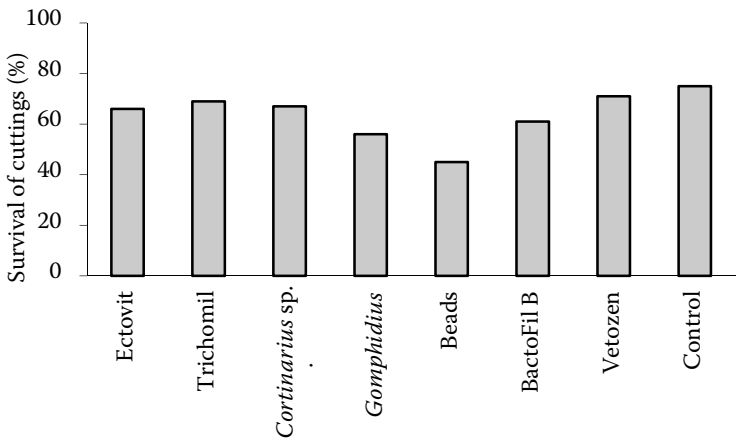

Fig. 1. The percentage survival of Norway spruce stem cuttings rooted for one growing season in a rooting substrate with the application of different commercial products (Ectovit, Trichomil, BactoFil B, Vetozen) or laboratory-produced alginate-beads fungal inoculum (*Cortinarius* sp., *Gomphidius glutinosus*). Treatment values are the means of survival of three blocks within treatment

Table 1. The percentage of ECM morphotypes and total ECM colonization (mean ± standard error) of Norway spruce stem cuttings rooted for one growing season in a substrate inoculated with commercial products or laboratory-produced fungal inoculum (*Cortinarius* sp., *Gomphidius glutinosus*)

Treatment	Ectomycorrhizal morphotypes						Total ectomycorrhizal colonization
	swollen, cylindrical or club-shaped			thin			
	dark brown, Brownish-black	light brown	another shades of brown	dark brown, Brownish-black	light brown	another shades of brown	
Cortinarius sp.	14.4 ± 2.5	18.0 ± 3.3	25.5 ± 2.9	1.7 ± 0.5	3.0 ± 0.6	28.3 ± 3.1	90.9 ± 2.1
Gomphidius	7.3 ± 1.7	15.4 ± 2.3	24.3 ± 2.6	0.8 ± 0.5	3.4 ± 1.2	28.4 ± 2.9	79.6 ± 3.1
Ectovit	6.4 ± 1.4	6.1 ± 1.6	19.1 ± 3.6	2.5 ± 0.9	3.4 ± 1.3	43.2 ± 3.5	80.6 ± 2.6
BactoFil B	15.7 ± 2.8	3.7 ± 0.8	9.9 ± 1.9	8.5 ± 1.9	5.9 ± 1.0	40.2 ± 3.6	83.9 ± 2.8
Trichomil	10.8 ± 2.1	7.9 ± 1.2	12.2 ± 2.1	4.8 ± 1.5	9.6 ± 2.1	39.7 ± 3.3	84.8 ± 2.6
Vetozen	6.2 ± 1.2	8.2 ± 1.5	18.8 ± 2.9	6.2 ± 1.5	5.6 ± 1.2	41.9 ± 2.6	86.7 ± 2.0
Beads	11.0 ± 3.1	16.2 ± 2.3	21.6 ± 3.4	0.1 ± 0.1	4.2 ± 1.8	32.8 ± 4.1	85.8 ± 2.1
Control	9.8 ± 2.5	23.4 ± 3.2	26.5 ± 2.9	2.5 ± 1.0	1.3 ± 0.7	21.8 ± 2.6	85.3 ± 2.6

beads without mycelium (45%). Neither the effect of commercial products nor the effect of laboratory-produced fungal inoculum on rooting % of cuttings was better than the control treatment. Relatively large differences in rooting % between blocks within any treatment were observed (*G. glutinosus* 27–77%, Vetozen 52–85%).

The effect of used products on the occurrence of ECM morphotypes and total ectomycorrhiza formation was not significant ($P = 0.05$). Total fungal colonization of cuttings ranged from 80% (*G. glutinosus* treatment) to 91% (*Cortinarius* sp.), 85% on average. The comparison of the ECM morphology and per cent of ECM colonization between treated and control cuttings revealed that the fungal inoculation and application of the other products did not lead to the formation of distinctive treatment-related macromorphological features of ECMs. The applied fungi failed to form specific ECMs and cuttings became mycorrhizal predominantly with naturally occurring indigenous fungi in all treatments. The ECMs were categorized to particular experimental morphotypes (Table 1). The common features of distinguished ECM morphotypes

Table 2. Growth parameters (mean ± standard error) of Norway spruce stem cuttings rooted for one growing season in a substrate inoculated with commercial products or laboratory-produced fungal inoculum (*Cortinarius* sp., *Gomphidius glutinosus)*

Treatment	Shoot number	Root number	Total shoot length	Total root length	Average shoot length	Average root length	Leading shoot length	Shoot dry weight	Root dry weight
			(cm)					(mg)	
Cortinarius sp.	3.9 ± 0.2	6.0 ± 0.3	11.7 ± 0.5	36.5 ± 2.3	3.1 ± 0.1	6.2 ± 0.3	3.3 ± 0.2	118.0 ± 5.6	26.8 ± 2.0
Gomphidius	3.5 ± 0.2	7.0 ± 0.4	10.3 ± 0.5	42.5 ± 2.6	3.0 ± 0.1	6.3 ± 0.4	2.9 ± 0.2	120.5 ± 6.1	33.5 ± 2.2
Ectovit	3.4 ± 0.2	7.1 ± 0.4	10.1 ± 0.5	47.7 ± 2.9	3.0 ± 0.1	7.2 ± 0.4	3.0 ± 0.2	112.0 ± 6.2	28.0 ± 1.8
BactoFil B	3.5 ± 0.1	7.7 ± 0.4	10.1 ± 0.4	43.8 ± 2.7	3.0 ± 0.1	5.9 ± 0.3	3.2 ± 0.2	107.8 ± 4.3	33.6 ± 2.1
Trichomil	4.0 ± 0.2	7.7 ± 0.4	11.4 ± 0.5	37.1 ± 2.6	2.9 ± 0.1	4.8 ± 0.2	3.2 ± 0.1	124.2 ± 6.5	28.5 ± 2.2
Vetozen	3.5 ± 0.2	7.8 ± 0.4	10.3 ± 0.5	44.5 ± 2.4	3.1 ± 0.1	5.8 ± 0.3	2.8 ± 0.2	114.9 ± 6.6	32.8 ± 2.6
Beads	3.5 ± 0.2	6.4 ± 0.4	10.3 ± 0.6	36.9 ± 2.4	2.9 ± 0.1	6.1 ± 0.3	3.1 ± 0.2	113.3 ± 7.3	29.2 ± 2.2
Control	3.4 ± 0.1	8.0 ± 0.4	10.3 ± 0.5	43.2 ± 2.8	3.1 ± 0.1	5.5 ± 0.3	3.0 ± 0.2	111.4 ± 5.6	30.1 ± 2.3

Table 3. Correlation coefficients of the linear dependence of growth parameters on the percentage of total ECM colonization of Norway spruce stem cuttings rooted for one growing season in a substrate inoculated with commercial products or laboratory-produced fungal inoculum (*Cortinarius* sp., *Gomphidius glutinosus)*

Treatment	Shoot number	Root number	Total shoot length	Total root length	Average shoot length	Average root length	Leading shoot length	Shoot dry weight	Root dry weight
					(cm)			(mg)	
Cortinarius sp.	–0.21	–0.01	0.03	0.16	0.23	0.23	0.07	0.05	0.06
Gomphidius	–0.14	–0.11	0.02	0.03	0.17	0.08	0.05	–0.03	0.03
Ectovit	–0.10	0.01	0.01	0.03	0.07	0.04	–0.05	0.08	0.01
BactoFil B	–0.16	–0.01	–0.11	–0.05	0.11	–0.12	0.23	0.11	–0.23
Trichomil	0.12	–0.33*	0.07	–0.23	–0.09	–0.02	–0.15	0.08	–0.05
Vetozen	0.08	0.16	0.16	0.07	0.09	–0.04	0.18	0.09	0.11
Beads	0.01	–0.07	–0.04	–0.24	–0.08	–0.18	–0.12	0.05	–0.27
Control	0.19	–0.02	0.1	0.2	–0.10	0.25	–0.15	0.08	0.19

*P = 0.05

(based on the colour and diameter) were simple or monopodial ramification, occasional loose mycelial mats, and no rhizomorphs. Thin ECMs of various shades between light and dark brown were the most abundant ECM morphotype (Table 1). Distinctly light and dark ECMs occurred on a small scale, with the exception of control cuttings, in which an increased abundance of swollen light ECMs was detected (23%).

The application of the test products to rooting substrate did not have any significant effect on growth characteristics of Norway spruce cuttings. The statistically most pronounced difference appeared in the root number of cuttings. Cuttings rooted in the substrate inoculated with alginate-bead inoculum of *Cortinarius* sp. formed the lowest number of roots (6.0), while those rooted in the control treatment showed the highest one (8.0). The inoculation with *Cortinarius* sp. was the weakest treatment also in other root parameters of cuttings, and oppositely, the best one in some shoot parameters (Table 2). The mean values of shoot number, length and dry weight indicate also a moderate stimulative effect of Trichomil application on the development of aboveground parts of cuttings.

A low degree, if any, of the dependence of growth characteristics on the percentage of ECM

Table 4. Chemical analysis of the photosynthetic apparatus of Norway spruce stem cuttings rooted for one growing season in a substrate inoculated with commercial products or laboratory-produced fungal inoculum (*Cortinarius* sp., *Gomphidius glutinosus)*

Treatment	Dry matter	C	N	P	K	Ca	Mg
		(%)			($mg.kg^{-1}$)		
Cortinarius sp.	90.62	53.85	3.29	1,662	9,106	13,688	5,788
Gomphidius	90.17	53.12	3.53	1,661	9,506	16,458	6,769
Ectovit	90.48	54.71	3.55	1,558	13,342	8,922	4,014
BactoFil B	91.07	53.37	3.40	1,695	9,747	13,190	5,624
Trichomil	90.56	54.99	3.51	1,599	9,417	12,741	5,521
Vetozen	90.85	55.37	3.62	1,616	9,405	13,218	5,839
Beads	90.90	53.36	3.32	1,835	8,860	15,689	6,262
Control	90.83	54.33	3.50	1,618	9,259	12,251	5,315

colonization analyzed by linear correlation was found (Table 3). The exception is a significant negative correlation between the root number and ECM formation of cuttings inoculated with the antagonistic fungus *Trichoderma* sp. (product Trichomil).

The values of dry matter, carbon and nutrient content in the photosynthetic apparatus of spruce cuttings were mostly equal in all treatments (Table 4). However, a higher concentration of K and lower concentrations of Ca and Mg were detected in cuttings rooted in the substrate amended with Ectovit than in those rooted in the other treatments.

DISCUSSION

The development of cuttings of coniferous trees inoculated by ECM fungi was investigated in studies of Stein and Fortin (1990) (*Larix laricina – Laccaria bicolor*), Stein et al. (1990) (*Picea mariana, Larix decidua – Laccaria bicolor, Suillus cavipes*), Parladé et al. (1999) (*Pseudotsuga menziesii – Laccaria bicolor, Melanogaster ambiguus, Rhizopogon subareolatus*), and Di Battista et al. (2002) (*Pseudotsuga menziesii – Laccaria bicolor*). Stein et al. (1990) were the first authors to report the enhanced rooting of coniferous stem cuttings (*P. mariana*) after an addition of solid ECM inoculum into the rooting substrate. In experiments of Stein and Fortin (1990), a higher number of roots and different rooting pattern were developed on hypocotyl cuttings of *Larix laricina* inoculated by *Laccaria bicolor* than on the uninoculated ones. ECM fungi can also be an appropriate tool of improvement of explant rooting and acclimatization in *in vitro* technologies (Normand et al. 1996).

The effect of inoculation of Norway spruce cuttings by ECM fungi on rooting, ectomycorrhiza formation, and growth of cuttings was assessed by Chmelíková and Cudlín (1990), Kuděla (1991), Chmelíková et al. (1992) and Repáč (1998, 2000, 2002, 2005, 2006). In any cases, inoculation resulted in the stimulation of shoot, root, and mycorrhiza formation. However, results were mostly inconsistent related to symbiotic partners, rooting substrate, inoculum type and application methods. Rooting % of Norway spruce cuttings ranged from 34 to 100 in these experiments. The relatively low rooting % of spruce cuttings in our experiment (45–75% according to treatments) was caused to a great extent by the absence of optimal environmental conditions for the rooting of cuttings in consequence of limited opportunities for the regulation of temperature, humidity and light conditions in a glasshouse. The values of these climatic parameters often exceeded critical allowable limits and resulted in increased desiccation of cuttings. Moreover, despite of the fungicide application a fluffy wadding-like mantle appeared on the surface of needles of some cuttings (the most probable mould pest was not identified), which occurred in a similar extent in each treatment. The infected cuttings were discarded from the experiment to localize disease diffusion.

Rooting % of cuttings inoculated by the laboratory-produced inoculum of *Cortinarius* sp. (67%), *Gomphidius glutinosus* (56%) and by commercial product Ectovit (66%) were slightly lower than those of the uninoculated ones (75%). The lowest rooting % in cuttings rooted in a substrate with the application of pure beads (45%) indicates possible unfavourable effects of beads on the rooting of cuttings also in treatments with beads containing the fungal mycelium. Repáč (1998, 2002) reported an adverse effect of fungal alginate-bead inoculum applied in a compact layer below the bases of inserted cuttings on rooting % of spruce cuttings. The author supposed harmful mechanical, chemical and/or microbiological effects of beads on rhizogenesis. Similarly in our experiment, despite of the regular dispersion of beads into the upper layer of rooting substrate, an unfavourable influence of beads on chemistry and microbial associations in substrate was very feasible. Rooting % in BactoFil B (61%), Trichomil (69%) and Vetozen (71%) treatments are similar to the other above-mentioned treatments. However, with regard to the extent and design of the experiment the differences were not tested statistically and thus the treatment effect on rooting % cannot be reliably qualified.

Chmelíková and Cudlín (1990), Kuděla (1991), Chmelíková et al. (1992) and Repáč (1998) reported a stimulative effect of the fungal inoculation of rooting substrate on ectomycorrhiza formation in Norway spruce cuttings. The fungal inoculation of rooting substrate in this experiment did not have any significant effect on the proportion of ECM morphotypes and total ECM colonization of spruce cuttings. The frequency and distribution of ECM morphotypes in particular treatments and high ECM colonization of roots in all treatments suggest the infection of roots by naturally occurring fungi. Artificially introduced fungi were not likely competitive enough with indigenous fungi to form ECMs, or they at best only contributed to their formation. The structure pattern of ECMs (mantle, Hartig net) indicated a high rate of ECMs in initial stages of development. On the other hand, dark brown and brownish-black

ECMs were either formed by certain morphotype-related fungi or they were later developmental stages of lighter brown ECMs induced by ageing. Progressive methods of identification and quantification of ECMs would have to be used for consistent and reliable analysis of ECM communities on roots of the evaluated cuttings (Hönig et al. 2000; Kennedy 2010). The application of ECM inoculum to a substrate does not guarantee that ECMs will develop on a host plant (Hönig et al. 2000; Repáč 2011). In addition to inoculum and inoculation pattern (type and age of inoculum used, inoculum dose, timing of inoculation, inoculum placement in the growing medium, etc. – Repáč 2011), the success of inoculation depends also upon interspecific and intraspecific host-fungus variation, environmental conditions, seedling production practices, and other factors (Kropp, Langlois 1990; Rincón et al. 2007). One of the likely reasons for the reduction or suppression of ectomycorrhiza formation by introduced fungi was the application of systemic fungicide, which was indeed needful for the restriction of pathogen activity in given environmental conditions of the glasshouse. According to Chmelíková et al. (1992) the mycorrhiza development is much slower in cuttings compared with seedlings of the same age, cultured under similar conditions. The reasons are decreased photosynthetic activity of needles under lower illumination and utilization of almost all available assimilates for plant regeneration. ECM formation can be stimulated by soil bacteria – plant growth promoting rhizobacteria (Höf-lich et al. 2001), especially in adverse conditions for the development of fungi (Brulé et al. 2001). The rooting substrate amended with bacteria in our experiment (product BactoFil B) did not affect the ECM development; the result coincides with findings of Shishido et al. (1996).

While some authors reported at least a partial stimulative effect of ECM fungi (Chmelíková, Cudlín 1990; Repáč 2005; Rincón et al. 2007) and soil bacteria (Shishido et al. 1996; Höflich et al. 2001; Jaloviar et al. 2008) on the growth of cuttings and seedlings of forest trees, the results of others are ambiguous (Kuděla 1991; Repáč 2007). In this study, neither the inoculation of rooting substrate by the laboratory-produced fungal inoculum of *Cortinarius* sp. and *G. glutinosus* nor the application of commercial products (Ectovit, Trichomil, BactoFil B, Vetozen) significantly stimulated the growth of roots and shoots of the spruce cuttings. Despite of the poorly developed root system, the *Cortinarius* and Trichomil inoculated cuttings reached slightly higher values of shoot parameters than those in the other treatments. With the exception of the negative dependence of root number on ECM colonization in Trichomil treatment, the biomass production of below and aboveground parts of the cuttings was not significantly ($P < 0.05$) correlated with ECM formation on roots.

The proposition that ECM symbiosis improves nutrient uptake and metabolic activity of trees is commonly accepted (Barnes et al. 1998). However, many authors (Eltrop, Marschner 1996; Ahonen-Jonnarth et al. 2003) pointed out the complexity of this association and great influence of many factors on the performance of symbiosis (tree species, substrate, fertilization, environmental conditions, etc.). Plant growth promoting rhizobacteria are able to facilitate the solubilization of mineral phosphates and other nutrients, retain more soil organic N and other nutrients in the plant-soil system, and enhance the release of nutrients (Hayat et al. 2010). Concentrations of nutrients in the photosynthetic apparatus of spruce cuttings were quite equal in all treatments of our experiment without any distinctive effect of fungi or products used. The exception was Ectovit treatment in which a higher concentration of K but a lower concentration of Ca and Mg than in the other treatments was detected. The differences in element contents should partly be caused by the activity of applied ECM fungi but more probably by the gelatinous form of application and by the composition of Ectovit product (peat, humates, ground minerals, water-retaining granules). Chemical analyses of the substrate and applied products (which were not done in this study) would help to explain nutrient content in the photosynthetic apparatus of plants in further experiments.

CONCLUSION

The deficiency of vegetative propagation of forest tree species by cuttings is insufficient quality of root systems, mainly a low proportion of short fine roots. Several previous studies showed that the inoculation of rooting substrate by ECM fungi and beneficial bacteria or the use of growth hormones can improve rooting %, root system quality, ECM formation and growth of stem cuttings of Norway spruce and other coniferous forest tree species. In this experiment, the application of alginate-bead laboratory-produced fungal inoculum and selected commercial microbial products to the rooting substrate did not have any significant effect on rooting %, morphology and abundance of ectomycorrhizas, and growth of roots and shoots of Norway spruce stem cuttings. The

application of Ectovit distinctly affected concentrations of K, Ca and Mg in needles as compared to the other treatments. Consistent regulation of temperature, humidity and light intensity of the rooting environment of cuttings, testing a wide range of inoculants (products), application techniques and doses, and a complex evaluation of cuttings are needed to support the ability of beneficial microorganisms to stimulate rhizogenesis and to improve the quality of Norway spruce rooted cuttings.

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