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Impact of mycorrhizal inoculation on spruce seedling: comparisons of a 5-year experiment in forests infested by honey fungus

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Abstract

Background and Purpose: *The eastern part of the Czech Republic is afflicted by a decline in spruce stands. Forests stressed by drought are subsequently infested by honey fungus. Treating the root systems of spruce seedlings with a mycorrhizal preparation could present a certain possibility for protection against honey fungus. Mycorrhizal preparations are used in forestry, nursery management, horticulture and agriculture for soil reclamatioEn, planting the slopes and shoulders of roads, planting urban green areas, cultivating medicinal plants, and so forth. In this paper, preliminary results of pilot tests are shown.*

Materials and Methods: *In the Nízký Jeseník area in the vicinity of the town of Skøipov, Norway spruce (Picea abies) seedlings were treated at five sites with the ectomycorrhizal preparation Ectovit. Four years after treatment, the treated and control seedlings were evaluated. Furthermore, seedlings were removed from the areas for laboratory evaluation of growth parameters and mycorrhizal characteristics.*

Results and Conclusions: *The results showed that artificial inoculation, or treatment of spruce seedlings with mycorrhizal preparation, positively supported the rooting, growth and development of spruce seedlings.*

INTRODUCTION

The eastern part of the Czech Republic is afflicted by a decline in spruce stands (*Picea abies* (L.) Karst.). Forest sites stressed by drought (*1*) are subsequently infested by honey fungus (*Armillaria* sp.) (*2*). The weakened trees are then colonized by bark beetles (*2*). In particular, such situation can be seen in Ostravská pánev (Ostrava Basin), Podbeskydská pahorkatina (Sub-Beskidian Hills), as well as in areas of higher elevations in the Silesian Beskids (*3*) and Hrubý Jeseník (High Jesenik) (Holuša, unpublished). Declining trees are actively searched out and subsequently felled and cleared. At certain locations, spruce has already vanished entirely from the tree species composition. Solution of this problem is to convert spruce stands to mixed deciduous forests (*2*).

In threatened locations, preference is given to planting deciduous trees and great effort is made to approximate natural tree species composition as much as possible. Although the main goal is to change tree species composition, treating the root systems of spruce seedlings with mycorrhizal preparation could present a possibility for protection against honey fungus.

One of the main goals of artificial inoculation is the successful survival of seedlings following transplantation and stimulation of their further efficient growth under stress conditions (*4*), which will lead to an improved health condition and resistance against various abiotic and biotic agents.

The rootlets would thus be colonized with mycorrhizal fungi and infestation by honey fungus prevented. Mycorrhizal preparations are used in forestry, nursery management, horticulture and agriculture for soil reclamation, planting the slopes and shoulders of roads, planting urban green areas, cultivating medicinal plants, etc. (*5–8*).

In this paper, we present the preliminary results of pilot tests in which we:

(i) compared the growth and development of Norway spruce seedlings treated with mycorrhizal preparation (inoculated) and those non-treated (control), and

(ii) assessed the colonization by mycorrhizal fungi in roots.

MATERIALS AND METHODS

Norway spruce seedlings were treated with the ectomycorrhizal preparation Ectovit® (a product from Symbiom s.r.o.) in Nízký Jeseník near Skřipov (49°49'12.325"N, 17°54'32.726"E) at 400–500 m.a.s.l. (*Fagetum typicum*). At present, Norway spruce dominates the tree composition. At 15 small clearings (< 0.5 ha) placed about 1 km in distance caused by logging of Norway spruce forest sections that had been dying out, a total of 50 two-year-old seedlings of the same dimensions were planted in five rows on 1–7 May 2004 and treated with the mycorrhizal preparation. Five rows were planted in parallel with seedlings not treated with the Ectovit preparation.

Ectovit is an inoculation material containing spores of ectomycorrhizal fungi (*Scleroderma* spp. and *Pisolithus* spp.), two solid carrier components (a mix of perlite and soft peat), and mixtures of natural materials supporting the occurrence of mycorrhizal symbiosis (seaweed extracts, natural sources of nitrogen, magnesium and potassium). The second component is a fluid medium in polyethylene sacks which contains sterile-cultured mycelium of other ectomycorrhizal fungi (*Laccaria* spp., *Boletus* spp., and others), chosen according to the species of the target tree (http://www.symbiom.cz/eshop/index. php).

In the period from 26 to 28 September 2008, the mortality rate and condition of the Norway spruce seedlings (height, thickness of the root crown) were evaluated. At each site, 20 seedlings (four from each row, always every second seedling) were measured. Furthermore, four treated (from rows 1–4, the second, fourth, sixth and eighth seedlings) and four control seedlings (40 in total) were removed from each test site for laboratory evaluation of their parameters (height of the aboveground portion, maximum root length, thickness of the root crown, dry weight of the aboveground portion and roots) and mycorrhizal characteristics.

The evaluation of mycorrhizal ratios was carried out using the standard method of identifying all active and non-active mycorrhizal tips (*9*). The main unit in determining the number of mycorrhizae was a root segment 5 cm long and of a diameter up to 1 mm. That was because the root fraction up to 1 mm in diameter tends to be the most important, most adaptable and most sensitively responsive to changes in stand conditions in terms of nutrients uptake from the soil (*9*). Twenty root segments on each root system were evaluated in this manner. The number of individual types of mycorrhizal tips was determined under a binocular magnifying glass according to the following diagnostic indications: nodules with a mature fungal mantle, a Hartig network (*10*) and high turgidity, devoid of root hair, smooth on the surface, and light colored are regarded as typical – these are ranked into the group »active mycorrhizae«. In contrast, nodules for which a significant loss of turgidity was evident, that were wrinkled on the surface, and that did not have a fungal mantle or Hartig network are ranked in the group »non-active mycorrhizae«. Some active mycorrhizal tips may be wrinkled and appear partially necrotic but at the same time can retain their physiological function. In such questionable cases, their sections were subjected to further examination microscopically.

The level of mycorrhizal relationships was then evaluated using two parameters: concentration of mycorrhizal tips and their percentage. The concentration of active and non-active mycorrhizae was calculated as an average value of the determined number of mycorrhizae relative to a 1 cm length of root. The percentage of mycorrhizae was calculated as the ratio of active and non-active mycorrhizae to the total number of all mycorrhizae found.

The root systems and aboveground portions of the removed seedlings were subsequently used to determine dry weight. The specimens were dried in a kiln at 105 °C and weighed with an accuracy of 0.01 g.

The measured parameters of the inoculated and control Norway spruce seedlings in the field and in the laboratory were tested using a two sample t-test. As with the numbers of survivor seedlings, for which the Mann-Whitney U test was used, the tests were performed using Statistica 8.0. programme.

RESULTS

The height of the Norway spruce seedlings measured in the field ranged between 99 cm and 140 cm. In the case of control seedlings, the average height was 103.5 cm, which was significantly lower than that for inoculated seedlings (133.5 cm) ($t = -8.01713$; $p < 0.01$; Figure 1). The height of the aboveground portion of the seedlings measured in the laboratory ranged from 70.3 cm to 96.5 cm. Again, in the case of control seedlings the average height (77.8 cm) was significantly lower than that for inoculated seedlings (90.6 cm) ($t = -2.67408$; $p < 0.05$).

Thickness of the root crowns of Norway spruce seedlings measured in the field ranged between 18.6 mm and

27.3 mm. Average value of 19.4 mm that was observed at control sites, was significantly lower than that of inoculated seedlings (26.1 mm) ($t = -9.08212$; $p < 0.01$; Figure 2).

The maximum root length for Norway spruce seedlings measured in the laboratory ranged from 25.6 to 41.3 cm. The average length was 29.6 cm in the case of control seedlings and 37.3 cm for inoculated seedlings ($t = -2.77942$; $p < 0.01$; Figure 3).

The percentage of active mycorrhizae ranged between 32% and 70%. The proportion of active mycorrhizae was 38% at control sites and 64% at inoculated sites (Figure 4). The percentage of active mycorrhizae reached significantly higher values in the treated Norway spruce seedlings ($t = -6.50379$; $p < 0.01$).

The average dry weight of the aboveground portion was 131.0 g for control seedlings and 182.0 g for inoculated seedlings, while the average weight of the root system was 26.0 g for control seedlings and 37.7 g for inoculated seedlings. But these results are not significant ($t = -1,6609$; $t = -1,79435$; $p>0.10$).

The rooting rate for control seedlings was lower than that of inoculated seedlings at all locations. Overall, 77% of control seedlings took root compared to 86% of inoculated seedlings. The average numbers are significantly different ($Z = -2.61116$; $p < 0.01$).

DISCUSSION

The positive impact of artificial inoculation of saplings with mycorrhizal fungi is evident in sterile cultures and in pot and field tests (*8*, *11–18*). The study of Castellano (*19*), in which the author analyzes the available results of mainly field inoculation experiments, provides a summary of the results of tests with mycorrhizal fungi. The statistical analysis conducted for all tree species and types of inoculum together demonstrated that the application of mycorrhizal inoculum very rarely leads to inhibition of tree growth. About half of the tests showed demonstrable stimulation. One of the reasons for ambiguity of results may be the inability to compare mycorrhizal trees with purely non-mycorrhizal control variants, which is very difficult to do in the field. Variability in the inci-

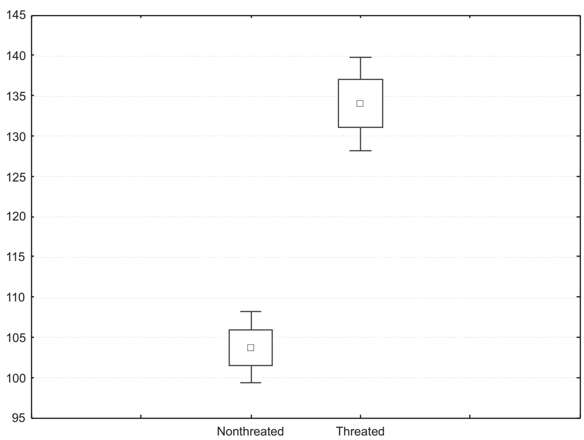

Figure 1. *Average height (±SE, ±SD) of the aboveground portion of spruce seedlings (cm).*

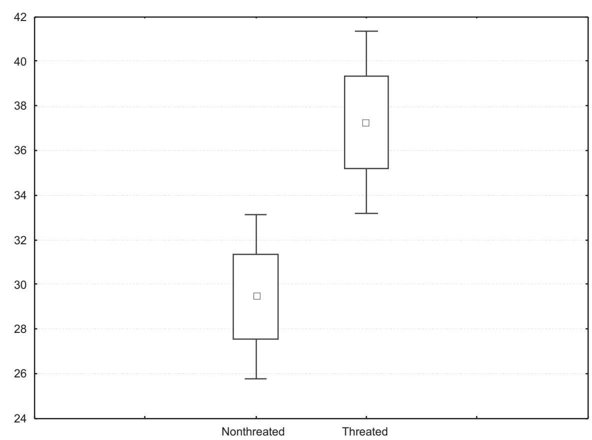

Figure 3. *Average maximum root length (±SE, ±SD) of spruce seedlings (cm).*

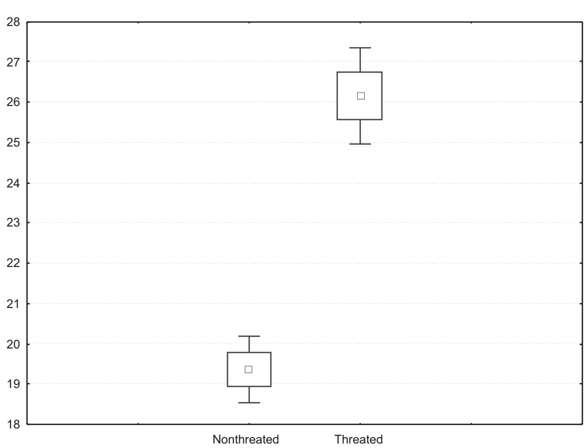

Figure 2. *Average thickness (±SE, ±SD) of the root crown of spruce seedlings (mm).*

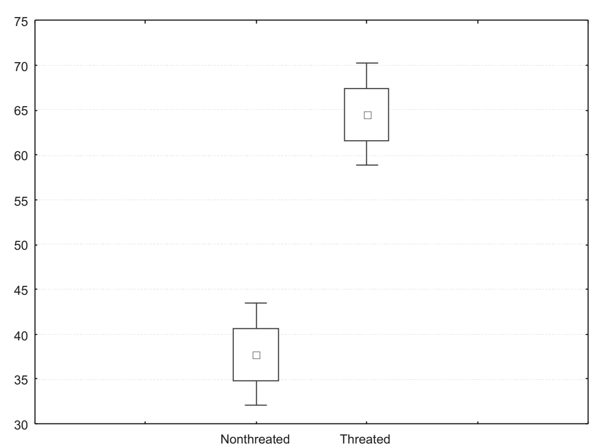

Figure 4. *Average percentage (±SE, ±SD) of active mycorrhizal nodules (AM) (%).*

dence of these micro-organisms under natural conditions, and depending on the soil conditions, as well as the impact of unrepeatable climatic conditions after several tested vegetation periods are the main causes for considerable differences in the results obtained experimentally with artificial inoculation of saplings and seedlings (*20*). The concentration of mycorrhizae is influenced primarily by long existing local conditions, while the percentage of mycorrhizae is noticeably more sensitive to such sudden changes as, for example, moisture stress, increased pollution, etc. (*21*, *22*).

In our tests, Norway spruce seedlings treated with the Ectovit preparation achieved larger dimensions, including seedling height, thickness of the root crown, root length (Figs. 1–3), and others even on forest sites with severe infestation by honey fungus (*Armillaria* sp.). Extensive studies conducted in Poland have also demonstrated statistically conclusive differences in the evaluated growth characteristics (height of the aboveground portion, annual growth, thickness of the root crown, and others) for Scots pine (*Pinus sylvestris* L.) seedlings inoculated with *Hebeloma crustuliniforme* (Bull.: Fr.) Quél. and *Laccaria bicolor* (Maire) P.D. Orton versus control seedlings (*8*). Increased height of the aboveground portion for mycorrhiza-treated seedlings were also evident in several other tree species including Norway spruce (*4*, *8*). In contrast, Tučekova *et al. (23),* in their initial results of a test of effectiveness of the mycorrhizal preparation Vambac with regard to the growth of seedlings planted in disaster clearings in Kysuce, indicate a weak positive impact of the preparation on the height of seedlings and thickness of the root crown. Tender, capillary roots developed favour rably after the first vegetation period following treatment of the Norway spruce with the mycorrhizal preparation (*23*).

In our experiment, statistically significant differences between average dry weight of the seedling and their roots were not confirmed in comparison with experimental inoculated Norway spruce trees. A mycorrhizal inoculum can significantly stimulate the growth of one-year pines and the doubling in the amount of root biomass (*24*).

Significant differences were demonstrated in the rooting of inoculated and control seedlings at all our test sites. Studies in Poland in the areas destroyed by fire showed that after five years 37% of seedlings inoculated with the mycorrhizal fungus *H. crustuliniforme* and 26% of those inoculated with the fungus *L. bicolor* survived, while all control seedlings died (*8*).

When assessing mycorrhizal characteristics, the concentration of active mycorrhizae and the percentage share of active mycorrhizae were demonstrably higher in inoculated seedlings. The results were significant even though, as opposed to artificial substrates, the soils at planted sites contain autochthonous ectomycorrhizal fungi and an entire range of micro-organisms with potential symbiotic relationships that could create uncontrollable interactions, influence the growth of seedlings, and perhaps even obscure the difference between the inoculated and control variants (*25*). In his tests with artificial inoculation of pines, Repač (*24*) also indicate that pine saplings to a significant extent tended to be colonized by indigenous, naturally occurring symbiotic fungi. Thus, mycorrhizae also spontaneously arise in non-inoculated controls which in some trials demonstrated even higher average values (*26*).

CONCLUSION

Artificial inoculation, or treatment of Norway spruce seedlings with a mycorrhizal preparation, supported their rooting, growth and development. The treated seedlings showed better results than control seedlings in majority of evaluation parameters. Based on the evaluation of this experiment, the method of artificial inoculating of seedlings in practical forestry can be recommended (after calculating economic costs) also in the area with severe infestation by honey fungus (*Armillaria* sp.). The further development and potentially better survival of Norway spruce specimens must be evaluated over a longer period. On the other hand, we must bear in mind that through artificial inoculation with mycorrhizae we carry out uncontrollable and unrestricted distribution into the environment of various inoculation preparations containing mycorrhizal inocula (*27*).

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REFERENCES

1. STANOVSKY J 2002 The influence of climatic factors on the health conditions of forests in the Silesian Lowland. *J For Sci 48*: 451–45
2. HOLUŠA J, LIŠKA J 2002 2002 Hypotéza hynutí smrkových porostů ve Slezsku (Česká republika). *Zpr Lesn Výz 47:* 9–15
3. ŠRAMEK V, SOUKUP F, SLODIČAK M, HELLEBRANTOVA K, LOCHMANOVA Z, NOVOTNY R, OCEANSKA Z, PEŠKOVA V, VICHA Z, VEJPUSTKOVA M, VORTELOVA L 2008 Chřadnutí lesních porostů na LS Jablunkov. Dílčí technická zpráva III a realizační výstup II, – Výzkumný ústav lesního hospodářství a myslivosti. *Strnady*, p 55
4. SZABLA K, 2005 Mikoryzacja sadzonek a efekty hodowlane w uprawach. [Autoreferát disertační práce]. Dyrekcji Generalnej Lasów Państwowych Warszawa, p 67
5. MARX D H, CORDELL C E, KENNEY D S, MEXAL J G, ARTMAN J D, RIFFLE J W, MOLINA R I 1984 Commercial vegetative inoculum of Pisolithus tinctorius and inoculation techniques for development of ectomycorrhizae on bare – root tree seedlings. *Forest Sci* (*Supp.) 25:* 1–101
6. KROPAČEK M 1989 Umělá mykorrhizace sadebního materiálu lesních dřevin. – Kandidátská disertace VŠZ Praha, Prag, p 145
7. REPAČ I 2000 Mykorízna symbióza lesných drevín a jej uplatnenie v škôlkárstve. Technická univerzita Zvolen, p 69
8. KOWALSKI S 2007 Ektomikoryzy. Nowe biotechnologie w polskim szkółkarstwie leśnym. Centrum Informacyjne Lasów Pañstwowych, Warszawa, p 398
9. PEŠKOVA V, SOUKUP F 2006 Houby vázané na kořenové systémy: Metodické přístupy ke studiu. Review. *Zprávy lesnického výzkumu, 51 (4):* 61–68

10. PETERSON R L, MASSICOTTE H B, MELVILLE L H 2004 Mycorrhizas: anatomy and cell biology. National research Council of Canada, Ottawa, p 173

11. CUDLIN P, MEJSTRIK V, SKOUPY J 1983 Effect of pesticides on ectomycorrhizae of Pinus sylvestris seedlings. *Plant Soil 71:* 353–361

12. DIXON R K, GARRET H E COX G S, MARX D H, SANDER I L 1984 Inoculation of three Quercus species with eleven isolates of ectomycorrhizal fungi. I. Inoculation success and seedling growth relationships. *Forest Sci 30 (2):* 364–372

13. HATCH A B 1937 The physical basis of mycotrophy in genus Pinus. *Black Rock For Bull* 6: 1–168

14. KROPAČEK K 1987 Testování granulovaného inokula za sterilních a polosterilních podmínek. pp. 65–71. *In:* Fellner R (*ed*) Ekologie mykorrhiz a mykorrhizních hub. Sborník referátů, DT ČSVTS, Pardubice

15. MORTIER F, LE TACON F, GARBAYE J 1989 Effect of dose and formulation of Laccaria laccata inoculum on mycorrhizal infection and growth of Douglas-fir in a nursery. *Agric Ecosystems Environ 28:* 351–354

16. MORTIER F, LE TACON F, GARBAYE J 1988 Effect of inoculum type and inoculation dose on ectomycorrhizal development, root necrosis and growth of Douglas fir seedlings inoculated with Laccaria laccata in a nursery. *Ann Sci For 45 (4):* 301–310

17. PARKE J L, LINDERMAN R G, TRAPPE J M 1984 Inoculum potential of ectomycorrhizal fungi in forest soils of Southwest Oregon and Northern California. *Forest Sci 30:* 300–304

18. THEODOROU C 1968 Inositol phosphate in needles of *Pinus Radiata* D. Don. and the phytase activity of mycorrhizal fungi. *Proc Congr Soil Sci 3:* 483–493

19. CASTELLANO M A 1996 Outplanting performance of mycorrhizal inoculated seedlings. *In:* Mukerji K G (*ed*) Concepts in Mycorrhizal Research. Kluwer Academic Publishers, Publishers, The Netherlands, p 223–301

20. PEŠKOVA V 2000 Mykorhizní inokulace, cesta, jak zlepšit ujímavost sazenic. *Lesn práce 79 (3):* 120–121

21. FELLNER R, PEŠKOVA V 1995 Effects of industrial pollutants on ectomycorrhizal relationships in temperate forests. *Can J Bot 73 (Suppl. 1):* 1310–1315

22. PEŠKOVA V 2006 Mykoflóra kořenových systémů lesních dřevin. Disertační práce – ČZU v Praze, Fakulta lesnická a environmentální, s. 84

23. TUČEKOVA A, LONGAUEROVA V, LEONTOVYČ R 2009 Poznatky z testovania mykorizovaného preparátu Vambac na smreku (*Picea abies* L.) v oblasti s dlhodobo zvýšeným stavom *Armillaria* sp. *In*: Holuša J, Pešková V (*eds*.) Mykorhíza v lesích a možnosti její podpory. sborník referátů, ČLS, VÚLHM, MZe, p 52–58

24. REPAČ I 2007 Poznatky z aplikácie symbiotických húb pri pestovaní semenáčikov borovice lesnej (*Pinus sylvestris* L.). *In:* Saniga M, Jaloviar P, Kucbel S (*eds*.) Obhospodarovanie lesa v meniacich sa podmienkach prostredia Zborník pôvodných vedeckých prác, technická univerzita Zvolen, p 163–170

25. GRYNDLER M, BALAŽ M, HRŠELOVA H, JANSA J, VOSATKA M 2004 Mykorhizní symbióza. O soužití hub s kořeny rostlin. – Academiae Praha, p 366

26. CAISOVA V 1994 Hodnocení mykorhizace sazenic smrku pichlavého (*Picea pungens*) po aplikaci tekutého a suchého inokula lakovky (*Laccaria proxima*). *VÚLHM 79:* 117–123

27. GRYNDLER M 2009 Mykorhizní symbióza – O soužití hub s kořeny rostlin. *In:* Holuša J, Pešková V (*eds*.) Mykorhiza v lesích a možnosti její podpory. Sborník referátů ČLS, *VÚLHM MZe:* 4 – 9