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Prethodno saopštenje *Preliminary report*

MYCORRHIZATION OF POPLARS (*Populus sp.*)

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Abstact: This paper presents results from world-wide and Serbian literature in the area of poplars inoculation (*Populus sp.*) with ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi. Responses of poplars cuttings and seedlings to inoculation by different ECM and AM fungi have been investigated *in vitro*, in pot and field experiments in Europe, North America and Asia. Different results have been obtained depending on examined fungal species and strain and poplar species, cultivar and clones, soil fertility, environmental conditions as well as on the trial duration. Results suggested that mycorrhizal symbiosis could improve growth of poplar trees, especially on disturbed sites. Incorporation of inoculation of *Populus sp*. with appropriate mycorrhizal fungi and selected bacteria (MHB and PGPB) into commercial nursery system could improve the establishment of poplars in various sites.

Key words: ectomycorrhiza, arbuscular mycorrhiza, poplars, inoculation

MIKORIZACIJA TOPOLA (Populus sp.)

Izvod: *U radu je prikazan pregled rezultata svetske i domaće literature iz oblasti inokulacije topola (Populus sp.) ektomikoriznim (ECM) i arbuskularno mikoriznim (AM) gljivama. Efekti inokulacije reznica i biljaka topola različitim ECM i AM gljivama su istraživani in vitro, u zemljanim kulturama i poljskim ogledima u Evropi, Severnoj Americi i Aziji. Dobijeni su različiti rezultati u zavisnosti od ispitivane vrste i soja gljive, vrste, sorte i klona topole, plodnosti zemljišta, uslova sredine, kao i dužine trajanja ogleda. Rezultati su pokazali da mikoriza može da poboljša rast topola, naročito na oštećenim staništima. Uključivanjem inokulacije Populus sp., odgovarajućim mikoriznim gljivama i odabranim bakterijama (MHB and PGPB) u komercijalnu rasadničku proizvodnju, moglo bi se poboljšati zasnivanje topola na različitim staništima.*

Ključne reči: *ektomikoriza, arbuskularna mikoriza, topole, inokulacija*

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1. MYCORRHIZA

Establishment, growth and survival of trees in most temperate and boreal forests depend on colonization of ectomycorrhizal (ECM) fungi. They successfully take water, organic and inorganic nutrients from soil and translocate them to the fine plant roots from which they take carbohydrates in turn (Smith and Read, 2008).

Seedlings colonized with appropriate fungal species and strains are favored in comparison to uncolonized ones in making contacts with water and nutrients as well as with other organisms in soil (Kraigher, 1996). It has been demonstrated by several investigators (Molina et al., 1992; Smith and Read, 2008; Quoreshi, 2008) that besides increased nutrient uptake, which is the most significant benefit of mycorrhiza, this symbiotic relationship offers numerous benefits which can be summarized:

- Enhance plant efficiency in absorbing water
- Reduce fertilizer and irrigation requirements
- Increase drought resistance
- Increase pathogen resistance
- Protect against damage from heavy metals and other pollutants
- Minimize various plant stresses
- Improve seedling growth and survival
- Improve soil structure by the extramatrical hyphal network
- Contribute to nutrient cycling processes
- Contribute toward carbon sequestration
- Increase plant diversity.

1.1 Inoculation

Mycorrhizal inoculation has been proven beneficial in a wide range of situations: for reclamation of disturbed sites, reforestation of clear cut areas and grasslands, reforestation after wildfire, and for introduction of exotic plant species (Marx 1991, 2002). Most research on inoculation with ECM fungi is based on two premises: any mycorrhiza on planting stock is better than none and some fungal species under some environmental conditions are more beneficial to trees than others (Marx, 1980).

Mycorrhizal fungal species varies greatly in their ability to support trees growing on different sites (Smith and Read, 2008). The degree of mycorrhizal responses on a reforestation site depends on the status of fungal colonization at planting site, persistence of introduced fungus, and other biotic and abiotic factors to planting site. Huge variation in response to inoculation often occurs from the above-mentioned factors as well as host-fungus compatibility, fungal effectiveness to site conditions, and efficiency of resident fungi (Grove and Le Tacon, 1993; Dodd and Thomson 1994).

1.1.1 Selection

Selection program of appropriate mycorrhizal fungi goes in two directions: in selection of hipervirulent strains from chosen natural habitats (for example in reforestation practices) and selection of strains that are capable of helping forest trees' seedlings to grow on extremely degraded or polluted soils (for example on mine or smelter impacted soils) (Kraigher, 1996).

In essence, the fundamental steps in any inoculation program are: (i) to identify and characterize the potential sites to be revegetated; (ii) collection, isolation, and identification of fungi; (iii) screening of fungi through *in vitro* selection procedures for identifying most promising strains; (iv) *In vivo* selection of selected fungal strain in association with host plants for larger inoculation program; (v) suitable inoculum production; (vi) development of large-scale inoculation program under commercial nursery environment, and inoculation of target indigenous plant species; (vii) outplanting of inoculated seedlings onto target sites for field trials, (viii) monitor plant growth, establishment and persistence of introduced mycosymbionts; (ix) finally, evaluation of the success of inoculation program (Quoreshi, 2008).

The initiation of a program of screening and selection of arbuscular micorrhizal (AM) fungi and ECM fungi for use as inoculants in agriculture, horticulture or forestry will depend on whether inoculation is more appropriate than manipulation of indigenous mycorrhizal populations. Most natural field soils and non-sterile nursery soils contain indigenous mycorrhizal fungi. A decision to introduce mycorrhizal fungi under these circumstances will also depend on the effectiveness of these indigenous fungi compared to possible inoculant fungi (Dodd and Thomson, 1994).

When indigenous fungi have a low colonization capacity, or have a high colonization capacity but are ineffective, plant growth might be increased by inoculating with effective mycorrhizal fungi. The greatest immediate potential for the use of mycorrhizal inoculants will be in areas where indigenous mycorrhizal fungi have been eradicated or drastically reduced by human influence or natural disturbance. These areas include mine sites, eroded sites, reforestation zones, low-input agricultural systems, fumigated soils (Dodd and Thomson, 1994).

In any ECM or AM inoculation program, the first step is to identify sites which will likely respond to inoculation. First of all this involves identifying the limitations to plant growth in particular soil and determine whether mycorrhizal fungi can relieve these growth restrictions.

Improved establishment and increased growth of inoculated plants with mycorrhizal fungi is commonly associated with higher rates of phosporus into the plant. Therefore the greatest potential for use of mycorrhizal inoculants will be in soils deficient in phosphorus for plant growth.

The initial stage in the selection of isolates involves defining the edaphic and climatic conditions where the fungi will be introduced. If the fungus is proved as tolerant to environmental extremes then ideally the isolation of mycorrhizal fungi should begin in such soils. In other to minimize the likelihood of incompatibility between fungus and soil, ideally inoculant fungi should be selected from the soil in which inoculated seedlings are to be planted (Dodd and Thomson, 1994).

1.1.2 ECM protocols

Isolation of ECM fungi can be obtained from sporophores, basidiospores, ectomycorrhizal rootlets and from sclerotia. Suitable isolation media include modified Hagem's agar (Modess, 1949 after Heinonen-Tanski and Holopainen, 1991) and modified Melin Norkrans' agar (Marx, 1969 after Heinonen-Tanski and Holopainen, 1991). The main difference between these media is that Melin-Norkrans'media contains more thiamine while Hagem's contain more ammonium salt and nutritionaly is more versatile (Heinonen-Tanski and Holopainen, 1991).

Langer et al. (2008) developed an ECM inoculation protocol specifically suitable for *Populus tremula*. Their results show that an exogenous supply of vitamins and micronutrients in modified Melin Norkrans Medium was a prerequisite for succesful mycorrhization of *P. tremula* with various *Laccaria, Hebeloma* and *Paxilus* isolates in Petri dishes.

After collecting and isolating a range of mycorrhizal fungi the next stage of the selection process is to screen these isolates for „effectiveness“. It usually means searching for isolates which increase nutrient uptake and therefore plant growth or isolates that develop extramatrical hyphal network which could have role in stabilizing eroded soils (Dodd and Thomson, 1994).

The selection of ECM fungi should begin with an initial screening of isolates to determine wheather they form mycorrhizas with the selected host. AM does not display the same degree of host-specificity. Synthesis of ECM fungi can be quickly assessed in axenic culture in laboratory. They can be also screened *in vitro* for other characteristics such as: their growth rate on the different media, response to different temperatures, pH and moisture, antagonism against selected patogens and production of secondary metabolites. Isolates should initially be compared in sterile soil or growth media that is representative of the field environment in which the isolates will be introduced. Effective mycorrhizal isolates can then be tested in natural soils under controlled conditions for their competitive ability with indigenous mycorrhizal population and other soil microorganisms. Isolates of AM and ECM fungi which prove to be effective in competition with indigenous mycorrhizal fungi must ultimately be tested for effectiveness in the field. These fungi must not only be able to compete with the indigenous mycorrhizal population under field conditions, but in some cases must also be able to persist and spread on roots over several years (Dodd and Thomson, 1994).

After *in vitro* screening and *in vivo* selection of the most promising fungal strain, inoculum production can be started. In forest nurseries are currently being used three types of inoculum to inoculate seedlings: vermiculite-peat based solid-substrate inoculum and liquid/mycelial slurry inoculum (these two are pure vegetative inoculums and contain mycelium) and spore inoculum (Quoreshi, 2008). Mycelium is used more often for inoculation then spores because ECM colonization from spores occurs only after hyphal fusion and the formation of dikaryons (Smith and Read, 2008).

Ma et al. (2008) investigated three methods of poplar seedlings inoculation with ECM fungi inoculum. In the first method after taking the seedling from the pot, solid inoculum was applied as base fertilizer at the bottom of each pot and the seedling was replanted. In the second they drilled and injected liquid inoculums into the holes around the root base. In the third method the seedlings were taken out, its roots were dipped in the inoculum slurry and then it was planted again. They obtained the best results with drilling and injecting liquid inoculums around root base.

1.1.3 AM protocols

Monoxenic cultivation of AM fungi involves the extraction of potential viable propagules from soils, surface sterilisation and optimisation of growth conditions for germination under aseptic conditions. This is followed by association of the propagules with a suitable excised host root for propagule production and recovery. Mass-produced propagules are then formulated in a utilizable form and stored before application to the target plant. Formulation is essentially a blend of microbial propagules with a range of carriers or adjuvants, to produce a material that can be effectively delivered to the target application.There are several mycorrhizal inoculum formulation. Glass beads have been used at the laboratories and expanded clay in commercial sector (Adholeya et al., 2005).

There are five main application technologies: broadcasting, in-furrow application, seed dressing, root dipping and seedling inoculation. In theory, the larger the number of AM fungal propagules delivered to the root zone at application, the faster the colonization of roots. Mass production of AM fungi has been achieved with several species, but *Glomus intraradices* remains the most promising (Adholeya et al., 2005).

There is currently an increasing interest in producing „mixed“ mycorrhizal inocula containing more than one or more mycorrhizal fungal species or even other organisms like beneficial bacteria or fungi. They may be an alternative to screening for broad range fungi adapted to conditions in the nursery, in the field or to extreme environmental conditions (Dodd and Thomson, 1994; Adholeya et al., 2005).

1.1.4 MHB and PGPB

Micorrhiza helper bacteria (MHB) are bacteria associated with mycorrhizal roots and mycorrhizal fungi that assist the mycorrhiza formation (Garbaye, 1994; Frey-Klett et al., 2007) and promote its functioning (Frey-Klett et al., 2007).

The presence of MHB as an ubiquitous group of micro-organisms and important for mycorrhizal symbiosis is suggested by the following findings: MHB have been found whenever they have been looked for, they are present in very different habitats, many of these bacteria seem to be closely associated with mycorrhizal fungi, and MHB can be found from taxonomically diverse bacterial groups (Tarkka and Frey-Klett, 2008).

It is assumed that not only single species but microbial communities have evolved to live in close association with mycorrhizal fungi and MHB may promote the mycorrhizal infection rate at different stages of the bacterium–fungus plant interaction.

Five major hypotheses explain the helper effect (Garbaye, 1994; Frey-Klett et al., 2007; Tarkka and Frey-Klett, 2008):

1. MHB promote germination of fungal propagules

It was shown by numerous experiments that the exudates of MHB often stimulate fungal spore germination.

2. MHB promote mycelial growth

Fungus–bacterium co-cultures are easily implemented and thus were often used as first indicators for the screening of MHB strains promoting hyphal growth. If MHB inoculation leads to increased mycelial biomass in the soil, the occurrence of root–fungus encounters should increase too, resulting in faster mycorrhization (Brule et al., 2001).

3. MHB modificate the mycorrhizosphere soil

The data from Brule et al. (2001) suggest that, with certain fungus–plant–substrate combinations, the MHB effect may only be observable when fungal growth is inhibited. Many of the soil microbes, including mycorrhizal fungi, produce toxic metabolites to suppress the growth of competitors. Helper bacteria could perhaps also suppress the production of toxic substances by soil microbes.

4. MHB help host recognition and changes in root system architecture

The recognition process between the host plant and the mycorrhizal fungus includes the reception of plant signals by the fungal mycelium, chemotrophic hyphal extension growth to the prospective infection site and characteristic changes in mycelia and hyphal morphology. Also, lateral root production can be positively influenced by MHB (Garbaye, 1994), probably due to the production of auxins or auxin-related substances by the bacteria.

5. MHB increase receptivity of the roots

The bacterium facilitates the colonization of the root system while growing in the rhizosphere prior to the contact between the mycorrhizal fungus and the host plant. This could occur through controlled production by the MHB of cell wall digesting enzymes, permitting the enhanced penetration of the roots by the fungal hyphae and easing their spread inside the root tissues. The suppression of plant defense response prior to fungal colonization could also potentially lead to enhanced mycorrhization.

MHB also promote the functioning of mycorrhizal symbiosys through atmospheric nitrogen fixation, nutrient mobilization from soil minerals and plant protection against root pathogens (Tarkka and Frey-Klett, 2008).

Specificity in MHB-mycorrhizal fungus interactions was already indicated in early studies, which described bacterial species that promote and others that were either neutral or inhibitory to mycorrhiza formation (Garbaye and Bowen 1987, 1989 after Tarkka and Frey-Klett, 2008). Because of their selectivity, MHBs might be an interesting, cheaper and safer alternative to soil fumigation.

For the applications of MHB in nurseries and in the field it would be desirable that the bacteria would have a strong short-term influence on mycorrhization, but a minimal effect on native microbial populations. Also, the effective dose of MHB that has to be used for increased mycorrhization varies between bacteria (Tarkka and Frey-Klett, 2008).

Plant growth promoting bacteria (PGPB) include representatives from very diverse bacterial taxa that exert a beneficial effect on plant growth.

PGPR may induce plant growth promotion by direct or indirect modes of action. Direct mechanisms include the production of stimulatory bacterial volatiles and phytohormones, lowering of the ethylene level in plant, improvement of the plant nutrient status (liberation of phosphates and micronutrients from insoluble sources; non-symbiotic nitrogen fixation) and stimulation of disease-resistance mechanisms (induced systemic resistance). Indirect effects originate for example when PGPR act like biocontrol agents reducing diseases, when they stimulate other beneficial symbioses, or when they protect the plant by degrading xenobiotics in inhibitory contaminated soils (Antoun and Prévost, 2005).

Several reports state that combined inoculation with PGPB and mycorrhizal fungi may yield synergistic positive effects on plant growth. Plant root-colonization with arbuscular mycorrhizal (AM) fungi can affect bacterial communities associated with the roots directly by providing energy-rich carbon compounds derived from host assimilates and transported to the mycorrhizosphere via fungal hyphae, by fungal induction of pH changes, by fungal exudates (inhibitory or stimulatory compounds) or by competition. Indirect effects of AM fungi can result from modification of soil structure or plant root exudates (Johansson et al., 2004).

However, antagonistic effects are often reported in the AM fungi-PGPR interactions. Positive interactions often result in plant growth improvement. Inoculation with both free living nitrogen fixing bacteria, such as *Azospirillum brasilense* or *Azotobacter* and AM fungi increases plant productivity. It is not clear whether the enhancement of plant growth is due to free nitrogen fixation or to the production of plant-growth promoting substances (Antoun and Prévost, 2005).

The combination PGPR and ectomycorrhizae have been studied for enhancing growth of tree seedlings in nurseries, but the effect of PGPR is either beneficial or detrimental for mycorrhization, depending on the study.

1.2 Poplars

In the last decade, poplars have become one of the most interesting trees for biotechnology. Besides their commercial importance, they combine many biotechnological advantages, such as: rapid growth, simple *in vitro* propagation and the existence of genetic transformation systems (Fladung and Ahuja, 1996). Also, poplar trees are good candidates for use in phytoremediation because they have deep roots, cycle large amount of water and grow rapidly (Newman et al., 1997). Poplars routinely form functional mycorrhizal associations with ectomycorrhizal (EM) fungi and arbuscular mycorrhizal (AM) fungi simultaneously (Molina et al., 1992) which can benefit them in establishing and growth in extreme conditions and makes them suitable for reforestation and reclamation purposes.

2. INOCULATION OF POPLARS

Responses of poplar cuttings and seedlings to inoculation by different ECM and VAM fungi have been investigated in pot and field experiments in Europe, North America and Asia. Different results have been obtained depending on examined fungal species and strain and poplar species, cultivar and clones , soil fertility, environmental conditions as well as on the trial duration.

Navratil and Rochon (1981) observed enhanced root and shoot development of poplar cuttings induced by *Pisolithus* inoculum. Cuttings from four poplar hybrids: *Populus* × cv. Northwest, *P.* × euroamericana cv. I-45/51, *P.* ×

euroamericana cv. DN-21, and *P.* × cv. robusta superba, were rooted in a medium inoculated with vermiculite-based vegetative inoculum of *Pisolithus tinctorius*. The addition of the inoculum to the medium resulted in an enhancement in shoot and root development of all four clones. All clones except one responded with significantly increased shoot length. At least two of the root characteristics measured were significantly increased by the addition of the inoculum in three of the clones: cv. Northwest, cv. I-45/51, and cv. DN-21. Cultivar variations in response to the inoculation were evident. As a cause of the enhanced shoot and root development are suspected hormonal exudates liberated by the mycelium of *Pisolithus*.

Cripps (2001) surveyed native mycorrhizal fungi associated with aspen on three soil types in the north-central Rocky Mountains and found 54 species of ectomycorrhizal fungi distributed in seven families: Amanitaceae, Russulaceae, Tricholomataceae, Cortinariaceae, Paxillaceae, Boletaceae, and Thelephoraceae which were screened for their ability to enhance aspen growth and establishment.

About half of the mycorrhizal fungi isolated grew in culture and fewer grew well enough to be tested. With the exception of *Inocybe lacera,* which produced 100% mortality in aspen seedlings, all plants inoculated with native mycorrhizal fungi were alive at the end of the experiment. Each mycobiont affected the morphology of aspen in a recognizable manner for the given conditions. Seedlings inoculated with nonnative fungi had a higher mortality rate (10–20%), and extreme leaf tips turned black.

Of nine selected isolates, all but one increased the biomass of aspen seedlings 2–4 times. Stem diameter, height, and number of root tips increased with inoculation of some fungi. The native species *Paxillus vernalis, Tricholoma scalpturatum, Hebeloma mesophaem, Thelephora terrestris,* and *Laccaria spp*. found most promising for further study. Although, was not native species on investigated area, *Pisolithus tinctorius* formed prolific mycorrhizae and stimulated plant growth.

Ma et al. (2008) examined mycorrhizal formation and effects of nine ectomycorrhizal fungi on the growth of poplar cuttings. The results showed that *Xrocomus chrysentero*, *Boletus edulis*, *Pisolithus tinctorius* and *Laccaria amethystea* formed clear ectomycorrhizal symbiosis with the poplar seedlings. Among these four ECM fungi *Xrocomus chrysentero*, had the greatest ability to develop mycorrhizae with all four poplar species. *Boletus edulis* showed a greater ability to form mycorrhizae with *Populus deltoides* Bartr cv. 'Lux' (Poplar I-69). *Pisolithus tinctorius* and *Laccaria amethystea* had relatively weaker abilities of colonization. The other five ECM fungal species, i.e., *Scleroderma luteus*, *Leccinum scabrum*, *Boletus speciosus*, *Calvatia craniiformis* and *Rhizopogen luteous* could not easily form mycorrhizae with poplar seedlings grown in sterilized substrates, but could do so in non-sterilized soil. This points out at significance of soil bacteria and other beneficial organisms for growth of mycorrhizal fungi.

Although growth-promoting effects were the best in the *Pisolithus tinctorius* and *Xrocomus chrysentero* groups all nine ECM fungi promoted length and basal diameter growth of the coppice shoots to different degrees which is in the accordance with previously described researches of Navratil and Rochon (1981) and Cripps (2001). Positive and beneficial effects of ECM fungi on their hosts are very well known (Molina et al., 1992; Smith and Read, 2008; Quoreshi, 2008).

Baum et al. (2000) inoculated a balsam poplar clone *Populus trichocarpa* by two ECM strains *Laccaria bicolor* and *Paxillus involutus* on two arable sandy soils with different organic matter and nutrient supply. Inoculation of poplars on the soil rich in organic matter and total nitrogen increased shooth length, biomass production, shoot: root ratio and total nitrogen uptake of the cuttings. On the other hand on the soil with lower organic matter and nutrient supply only the shoot: root ratio and the nitrogen nutrition were improved. These results showed the importance of soil type for the relations between ectomycorrhizal fungi and their partner.

Also, Baum et al. (2002) investigated growth response of *Populus trichocarpa* to inoculation by the ectomycorrhizal fungus *Laccaria laccata* in a pot and a field experiment. The inoculation by *L. laccata* caused significantly increased shoot length and leaf potassium concentration of the poplar clone after the first growing season. During the second growing season, only the leaf potassium concentrations were increased compared to the non-inoculated control plants. The density of AM spores in the soil and the leaf nitrogen, magnesium and calcium concentrations were significantly reduced after inoculation. However, after the second growing season there were no longer significant differences in the ECM colonization and shoot lengths of inoculated or non-inoculated poplar cuttings. The results indicated that inoculation can be successfully used to increase ECM colonization and growth rates of *P. trichocarpa* in the first growth period. This could increase the resistance of the cuttings to soil-borne pathogens and their competitiveness for nutrients and space against weeds.

The field performance of four poplar clones (Walker poplar, Manitou poplar, Balsam poplar, and White aspen) inoculated with different inoculation treatments was evaluated 3 years after outplanting in work of Quoreshi et al. (2008). The inoculation treatments were: six ECM fungal species, *Hebeloma longicaudum*, *Laccaria bicolor*, *Paxillus invulutus, Pisolithus tinctorius, Rhizopogon vinicolor, Suillus tomentosus*, an endomycorrhizal fungus *Glomus intraradice*. The effects of nursery inoculation of different poplar clones, aspen and balsam poplar were very limited. Five years after outplanting, in general, different inoculation treatments had no remarkable effect on growth and survival of all the plant species tested compared to control. The poor growth response observed in this trial is probably related to several factors. Two most important factors could be: the lack of more host-specific and ecologically adapted fungal strains for these poplar species and lack of competitiveness of introduced fungi with indigenous mycorrhizal fungi. This work points out on the significance of plant-fungus compatibility and fungal effectiveness to site conditions and competitiveness to resident fungi.

Quoreshi and Khasa, (2008) inoculated aspen and balsam poplar seedlings with six species of ectomycorrhizal fungi *(Hebeloma longicaudum, Laccaria bicolor, Paxillus involutus, Pisolithus tinctorius, Rhizopogon vinicolor,* and *Suillus tomentosus)*, one species of endomycorrhizal fungus (*Glomus intraradices*), two species of bacteria (*Agrobacterium sp.* and *Burkholderia cepacia*), treated with a growth hormone (SR3), and co-inoculated with a combination of *Paxillus* and *Burkholderia*. The seedlings were grown in a greenhouse under three different fertility regimes. Bacteria alone did not affect seedling growth and nutrition as observed when co-inoculated with ectomycorrhizal fungus. The biomass and root collar diameter of aspen and balsam poplar were significantly increased when adequate mycorrhizas are formed and more prominent when co-inoculated with *P. involutus* and *B. cepacia* and grown at the 67% fertilizer level. Except for *R. vinicolo*r and *S. tomentosus*, the other four species of ectomycorrhizal fungi and *G. intraradices* formed symbiotic associations with both plant species. Both ectomycorrhizal and endomycorrhizal colonization were observed at all fertilizer levels and fertilizer applications did not affect the colonization rates. Nitrogen and phosphorus concentrations were significantly improved in both aspen and balsam poplar compared to control only when co-inoculated with *P. involutus* and *B. cepacia*. However, plant net nitrogen uptake (content) increased significantly in all successful inoculation treatments and co-inoculated treatment when compared with control.

These results suggested that mycorrhizal symbiosis improves the utilization of the absorbed nutrients and hold promise for incorporation of inoculation of *Populus sp*. with appropriate mycorrhizal fungi and selected bacteria into commercial nursery system to improve the establishment of *Populus* in various sites.

Gehring et al. (2006) examined the influence of environment and host crosstype on the ECM and AM fungi colonization of cottonwoods (*Populus angustifolia* and natural hybrids) by comparing levels of colonization of trees growing in common gardens that differed in elevation and soil type. They conclude that environment, particularly soil moisture, has a larger influence on colonization by AM versus ECM fungi than host genetics, and suggest that environmental stress may be a major determinant of mycorrhizal colonization in dually colonized host plants.

It was found that although some fungal species could not form clear mycorrhizae with poplar seedlings, they did promote seedling growth to some extent. This indicates that the growth promoting effect was not only owed to mycorrhizal formation which changed root morphology and enlarged the absorption area, but also due to some extra-cellular substances secreted by the ECM fungi, which were supplied to the seedlings directly or improved the rhizosphere environment to increase absorption, thus promoting seedling growth (Ma et al., 2008).

Similar results obtained Cripps (2001) who observed substantially increased biomass of poplar plants with addition of some fungi, but in most cases only a low percentage of roots were colonized in the given time period. Measured percentage of mycorrhizal roots was not directly correlated with increases in aspen biomass, stem diameter, and height. She also thought that it could be a result of high efficiency nutrient transfer through a small number of individual mycorrhizae or due to pre-mycorrhizal effects such as release of IAA.

2.1 Potential use of ECM and AM fungi inoculated poplars in forestry, reclamation, reforestation and phytoremediation

Outplanting of inoculated material may be a key element for enhanced establishment onto agricultural or disturbed soils where mycorrhizal inoculum is low and/or ineffective and fungal dispersal is insufficient. Addition of specific mycorrhizal fungi substantially increase host plant survival, growth and biodiversity.

Moreover, co-inoculation of specific ectomycorrhizal and arbuscular mycorrhizal fungi and other beneficial bacteria might show synergistic effects on hybrid poplar growth (Dodd and Thomson, 1994; Marx, 2002; Smith and Read, 2008; Quoreshi, 2008).

Khasa et al. (2002) investigated occurrence of the most common mycorrhizal types of selected poplar clones introduced in to previously cleared agricultural or disturbed sites in the province of Alberta and found variable degrees of colonization by both ectomycorrhizal and arbuscular mycorrhizal fungi, suggesting differential host receptivity (susceptibility). This comfirmed the potential use of selected strains of both ectomycorrhizal and arbuscular mycorrhizal fungi for reforestation and reclamation.

Mycorrhizal fungi can help their plant partner to establish and grow in extreme conditions, where plant could not survive alone. It was found that *Paxilus involutus* attenuated NaCl-stress in salt-sensitive hybrid poplar *Populus x canescens* and whole plant performance was affected positively by the fungus. Inoculated plants had greater total biomass and leaves accumulated less Na^+ (Langenfield-Heyser et al. , 2007).

Also, mycorrhizal fungi have very important role in the phytoremediation processes in which their partners take part. In diesel contaminated soils, colonization of hybrid poplar with ECM fungus *Pisolithus tinctorius* (Pers.) increased fine root production and whole plant biomass, N and P contents in leaves, but inhibited removal of total petroleum hydrocarbons from the soil compared to uninoculated treatments. (Gunderson et al., 2007)

Poplars are very often used in phytoremediation of heavy metals (Katanić et al., 2009) and their partners ECM and VAM fungi may be of the great significance in such processes.

Poplars and willows cuttings were planted in copper and iron mine tailings and after two years incidence of mycorrhizae was examined. Results showed that no mycorrhizal development occurred on roots of these tree species in the copper tailings, but roots in the iron mine tailings developed extensive ectomycorrhizae and trees showed good growth. The poor growth of non-mycorrhizal willows and poplars found in the copper tailings suggested that mycorrhizae play an essential role in the development of these trees on such sites. Establishment of vegetation on similar areas has depended largely upon the successful development of mycorrhizae. In areas where soil lack appropriate mycorrhizal fungi reforestation without inoculation is generally unsuccessful (Harris and Jurgensen, 1977).

Todeschini et al., (2007) grown two registered clones of poplar, Villafranca (*Populus alba*) and Jean Pourtet (*P. nigra*), in soil contaminated with copper. Some of the plants were pre-inoculated with arbuscular mycorrhizal (AM) fungi *Glomus mosseae* (Gerd. and Nicol.) and *G. intraradices* (Schenck and Smith). Copper effects on plant growth were evaluated, as well as the role of AM fungi in alleviating metal stress. Two clones showed several differences in relation to copper pollution and AM symbiosis, confirming the importance of accurate plant selection for phytoremediation purposes. Accumulation of copper in roots suggested that poplar is suitable for phytostabilization strategies in the presence of this metal. Also, pre-inoculation with AM fungi improved growth conditions in copper-treated plants, suggesting that improved mineral nutrition could be responsible for the alleviation of copper stress.

Sell et al. (2005) investigated the possibility of enhancing phytoextraction of cadmium by poplars (*Populus canadensis*) in association with ectomycorrhizal fungi *Hebeloma crustuliniforme, Paxillus involutus and Pisolithus tinctorius*. The association of *P. canadensis* with *P. involutus* led to a highly significant increase of Cd concentrations, in particular in the leaves. Compared to the control this enhancement was of nearly 100%. The fungi also significantly enhanced the translocation from the roots to the leaves and the total cadmium extraction. The presence of the two other fungi also led to significantly enhanced cadmium accumulation in the leaves.

Possible role of mycorrhizal fungi in reclamation processes was studied by Obase et al. (2009) who examined mycorrhizal synthesis of four ectomycorrhizal fungi: *Laccaria amethystina*, *Hebeloma mesophaeum*, *Thelephora terrestris* and *Tomentella* sp. on *Populus maximowiczii* seedlings potted in volcanic debris in a controlled growth chamber. The effects of ECM colonization on host plant growth were larger seedling height and biomass in the inoculated seedlings than in the control, although the effects of inoculation varied with the ECM fungus. All inoculated ECM fungi promoted seedling growth in both height and weight, contributing to the establishment on this disturbed site. The growth promotion of host plant by ECM colonization is possibly related to increased efficiency of nutrient acquisition, such as nitrogen and phosphorus, from volcanic debris. These findings pointed out on possible use of these fungi in reclamation processes of disturbed sites with low nutrient content.

Each aspen stand hosts a diverse community of mycorrhizal fungi as determined by soil type, age of the aspen stand, geographic region, and other edaphic and historical factors. Young aspens in pioneering situations, such as post-fire and smelter sites and previously unforested land, depend on “early stage” mycorrhizal fungi such as *Inocybe, Laccaria, Hebeloma, Thelephora,* and *Paxillus* for establishment and health. Many of these “weedy” species of fungi are most likely to be of use in mined-land reclamation, and they increase aspen biomass, height, and stem diameter *in vitro* (Cripps, 2001).

In older aspen stands, “late stage” mycorrhizal fungi make up a large part of the mycoflora, and these are species more closely allied with aspen than with other tree species. Soil type and other factors can affect the “succession” of mycorrhizal fungi. Management practices could apply selective pressures that promote certain species of mycorrhizal fungi, possibly to the exclusion of others, with long-term unintended consequences (Cripps, 2001).

Cripps (2003) initiated investigation of the use of native mycorrhizal fungi for enhancement aspen establishment on smelter-impacted sites. Typically a commercial fungal inoculum was added to trees on disturbed areas, but inherent problems are: spread of exotic fungi, and use of expensive generic fungi which are not site/host specific. One solution was use of native fungi adapted to a particular tree species, soil type and climatic region.

The first goal was to catalogue ectomycorrhizal fungi that occur with aspen in copper smelting area and the next step was to select mycorrhizal fungi that might have potential for use in reforestation by aspen in heavy metal contaminated soils using greenhouse studies and field trials.

Thirty species of native fungi were reported on these sites, half of which grew under laboratory conditions. A few grew well enough to warrant further interest as inoculum, including: *Laccaria proxima, Tricholoma flavovirens, Tricholoma populinum, Scleroderma cepa,* and *Paxillus vernalis*. After that, inoculum was being developed for use in greenhouse and field studies with aspen.

The mycorrhizal fungi recorded on smelter-impacted sites appeared to tolerate stressed conditions, and particular strains could be valuable for use in reclamation. These are very valuable information for further studies.

According to Kraigher et al., (2007) the functional compatibility and stress tolerance of ectomycorrhizal types is species specific, and therefore the information on the ectomycorrhizal community structure can also be applied as tools for bioindication of pollution stress in forest soils.

There are only few researches from this area that have been done in Serbia, yet.

Galić et al. (2007) investigated the influence of mycorrhizae application on production of high-yield poplar varieties seedlings. Research related to the effects of treatment by commercial mycorrhiza preparations Ectovit, Rhodovit (preparations Symbio-m Ltd., Czech Rep.) and their combination on growth of four high-yield poplar clones of *Populus deltoides* and one variety of *Populus x euramericana*. The study results indicate that mycorrhized cuttings had the same or the better survival in all the study clones compared to the control. The application of the preparation Ectovit and Rhodovit resulted averagely in the first class planting stock of all the study clones. The combination of the preparations Ectovit and Rhodovit produced averagely the first class planting stock only of the clone *Populus x euramericana*. These results are in the accordance with previous findings that addition of specific mycorrhizal fungi can substantially increase host plant survival and growth.

Also, first identification of ectomycorrhizal types on poplars in Serbia was made by Katanić et al. (2008a) and six types of ECM fungi were found. Investigation of diversity of ECM types on poplars is continued in order to define which ECM fungi would be the most appropriate for inoculation of poplar seedlings that are to be planted on various sites.

2.2 Commercial application of ECM and AM fungi inoculums

The major barrier to development of cheap and easy inoculant technique is that AM inoculums currently have to be grown with plants. The plant-based inocula now available are quite diverse and require different methods of application. Spores, hyphae, root fragments which are used as the source of inocula are added to different carriers, resulting in a wide range of formulations (Smith and Read, 2008).

The suitability of these inocula for different application depends on the identity of the main AM propagules and on their ability to retain infectivity during storage and to persist in soil or roots from year to year, as well as on methods available for application. One promising approach is incapsulation of AM roots, containing high densities of fungal vesicles in alginate beads.

According to Smith and Read (2008) and Quoreshi (2008) AM inocula should meet reasonable standards such as:

- inoculum must initiate colonization in the root systems of plant species that are able to form mycorrhizas, at the doses recommended by the suppliers,

- it must not contain pathogens or other agents that could reduce plant growth,
- it must have reasonable shelf life when stored under recommended conditions
- the products should decrease the need for fertilization application, increase plant growth, flowering, yields or tolerance to disease or pollutants.

At present, routine inoculation in broad scale, highly developed farming system is not realistic, because of the expanse of production and uncertainties relating to the competitive ability of inoculants fungi in field situations.

Inoculation with ECM fungi can have benefits at two stages of the timber production systems: in the nursery itself and after outplanting to the field. A lot of experiments are focused on advantages to be gained from the production of well-developed seedlings that, with their fungal symbionts, will become successfully established in the field. Experience of the use of inoculated seedlings has indicated that responses to ECM colonization are often greatest under the most extreme conditions especially drought, metal contamination and pathogens.

Prerequisites for the widespread use of ECM inoculation programs are the selection of fungal symbionts and development of methods for the large-scale inoculum production.

In order to eliminate weeds, pathogens and other symbiotic fungi which are potential competitors, seed beds or potting mixes are routinely fumigated before inoculation. Even so, re-invasion of fumigated soil by spores of naturally occurring fungi (*Telephora terrestris*, particularly) naturally occurs within days and is required that inoculated fungi are able to colonize root quickly.

Outplanting trials in several regions of the world indicate that increased growth in nurseries may not be correlated with improved performance in the field and that inoculant fungi may persist only a few years after outplanting, before being supplanted by naturally occurring fungi. Nevertheless, it is quite likely that early benefits accrue from dependence of young trees on uptake of nutrients from soil via their ECM symbiont. Various commercial inoculums formulations and inoculation techniques have been developed for use in seedling production systems. A major challenge is the selection of appropriate fungi for inoculation programs. It is based on their performance as symbionts for the plant species in nursery production and on their likely survival and competitiveness at field sites. Unfortunately, it appears that many of those fungi selected to achieve optimal colonization in the nursery are poor competitors in the field, especially when outplanting sites contain indigenous populations of mycorrhizal fungi.

There are a number of possible explanations for the common failure of inoculation to produce beneficial effects at outplanting sites. Probably one of the most important is the inability of introduced inocula to persist on the roots of planting stock after their transfer from the nursery to the field. Also, soil conditions on outplanting-sites differ from ones in nurseries and containers. Lifting, storage and transport of seedlings can reduce the vigor of the fine roots and their associates. These treatments are likely to favor replacement of introduced fungi by indigenous ones.

Most strongly beneficial effects of inoculation have been observed when plants are transferred to disturbed or treeless sites where inoculum potential of indigenous fungi is low. Improvements in survival and increases in yields are most marked if soil is contaminated with heavy metals (Smith and Read, 2008).

It can be suggested that indigenous fungi from disturbed areas are most suitable for inoculation of plants that are going to be planted in such areas. Further studies are needed to be done in order to find out more about this, because interactions between fungi, plants and their environment are very complex.

3. LITERATURE

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Sažetak

MIKORIZACIJA TOPOLA (Populus sp.)

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Zasnivanje, rast i preživljavanje drveća u većini umerenih i borealnih šuma zavisi od kolonizacije ektomikoriznim (ECM) gljivama. Sadnice naseljene odgovarajućim vrstama i sojevima gljiva su u prednosti u poređenju sa nenaseljenim u stvaranju veza sa vodom i hranljivim materijama kao i sa drugim organizmima u zemljištu.

Sađenje inokulisanog materijala može da bude ključni element za unapređivanje zasnivanja drveća na poljoprivrednim ili oštećenim zemljištima gde mikoriznog inokuluma ima malo i/ili je neefikasan i širenje gljiva je nedovoljno. Dakle, inokulacija mikoriznim gljivama je dokazana kao korisna u mnogim situacijama: za obnovu oštećenih staništa, pošumljavanje lokaliteta nakon totalnih seča i travnih površina, pošumljvanje nakon prirodnih požara, kao i prilikom unošenja egzotičnih biljnih vrsta. Program odabiranja odgovarajućih gljivnih vrsta se odvija u dva smera: odabir hipervirulentnih sojeva sa odabranog prirodnog staništa i odabir sojeva koji mogu da pomognu sadnicama drveća da rastu na krajnje degradiranim ili zagađenim zemljištima. Takođe, ko-inokulacija specifičnim ektomikoriznim i arbuskularno mikoriznim gljivama i korisnim bakterijama (bakterije koje pomažu mikorizu (MHB) i bakterije koje podstiču rast biljaka (PGPB)) može da ima sinergističke efekte na rast topola.

Efekti inokulacije reznica i biljaka topola različitim ektomikoriznim i arbuskularno mikoriznim (AM) gljivama su istraživani in vitro, u zemljanim kulturama i poljskim ogledima u Evropi, Severnoj Americi i Aziji. Dobijeni su različiti rezultati u zavisnosti od ispitivane vrste i soja gljive, vrste, sorte i klona topole, plodnosti zemljišta, uslova sredine, kao i dužine trajanja ogleda..

Iskustvo nastalo upotrebom inokulisanih sadnica pokazuje da su efekti kolonizacije topola mikoriznim gljivama često najizraženiji u najekstremnijim uslovima, naročito uslovima suše, zagađenja teškim metalima i patogenima. Moglo bi se sugerisati da su autohtone gljive sa oštećenih staništa najpogodniji izbor za inokulaciju topola prilikom pošumljavanja takvih i sličnih lokaliteta.

Uključivanjem inokulacije topola, odgovarajućim mikoriznim gljivama i odabranim bakterijama, u komercijalnu rasadničku proizvodnju bi se moglo da poboljšati zasnivanje topola na različitim staništima.